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# THE EFFECTS OF TEMPERATURE ON BLEEDING TIME AND CLOTTING TIME IN NORMAL VOLUNTEERS

BY

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## ABSTRACT

BACKGROUND: Bleeding time as a predictor of the potential for a bleeding disorder has been debated. The bleeding time measurement is known to be influenced by platelet count, mean platelet volume, platelet mass, von Willebrand's factor, factor VIII clotting protein, capillary integrity, vessel reactivity, and certain drugs, as well as by mechanical events associated with local perfusion pressure, resistance and blood flow. A bleeding time of less than 10 minutes has been arbitrarily established as a normal value.

In this study , the effects of temperature on bleeding time and clotting time were evaluated in healthy volunteers. Previous studies in humans subjected to extracorporeal bypass surgery and in baboons revealed correlations among increased bleeding time, reduced local skin temperature, and a reduced thromboxane B2 level in shed blood collected at the template bleeding time site.

METHODS: Forty-one (41) normal volunteers (19 males and 22 females) were subjected to local warming and cooling of the forearm to achieve local skin temperatures of +38C, +35C, +32C, +29C, +26C, +23C, and +20C or +37C, +32C, +28C, and +22C. Bleeding times were measured, and thromboxane B2 was measured in shed blood collected at the template bleeding time site. Thromboxane B2 is the stable metabolite of thromboxane A2 which vasoconstricts the blood vessels and aggregates the platelets at the bleeding time site. The total hemoglobin on the filter paper was recovered and measured, and the measurement was correlated to the bleeding time. Thromboxane B2 levels also were measured in the serum and plasma obtained from the venous blood collected from the antecubital vein of the locally cooled and warmed forearm.

Twenty-four (24) in vitro studies were done in blood collected from normal volunteers without altering the temperature of the forearm. In vitro clotting times were measured at +37C, +32C, +28C, and +22C and the serum was collected at the end of the clotting time

or at 30 second intervals during the clotting of the blood for serum thromboxane B2 measurement in both agitated and non agitated blood.

We also studied the in vitro effects of temperature on the measurement of PT, PTT, factor V, and factor VIII and the in vitro effects of temperature on platelet aggregation and on thromboxane B2 production in platelet rich plasma isolated from venous blood collected from the antecubital vein after stimulation with a combination of arachidonic acid (AA) and adenosine diphosphate (ADP) or with ristocetin alone.

RESULTS: A reduction in local skin temperature from +38C to +20C was associated with a significantly increased bleeding time, a significantly decreased thromboxane B2 level in the shed blood collected at the bleeding time site, and a 15% reduction in the rate of thromboxane B2 production in the shed blood collected at the template bleeding time site. A reduction in the temperature of the forearm from +38 to +20C was associated with a significant reduction in the serum thromboxane B2 level in clotted blood obtained from the

antecubital vein. The partial thromboplastin time and the prothrombin time were significantly increased when measured at +22C compared to +37C.

A prolonged bleeding time was associated with a significant increase in the total amount of hemoglobin recovered from the filter paper used to collect the blood from the bleeding time incision.

CONCLUSIONS: In healthy human volunteers, a reduction in skin temperature of the forearm was associated with a significant increase in bleeding time, a significant reduction in the thromboxane level in the shed blood obtained at the template bleeding site and a significant reduction in the serum thromboxane B2 level in the clotted blood obtained from the cooled forearm. These data indicate the importance of measuring local skin temperature at the time the bleeding time is performed, and of correcting the bleeding time measurement for the skin temperature. The bleeding time should be corrected for the skin temperature to distinguish the effects of the individual's platelets, clotting proteins, and vessel reactivity, as well as of drugs, anesthesia, and blood products on the bleeding time.

The temperature at which blood is clotted also affects clotting time: a reduction in temperature was associated with an increase in clotting time and a reduction in serum thromboxane B2 obtained from the clotted blood. Each degree centrigrade reduction in temperature was associated with approximately a 15 percent reduction in the rate of thromboxane B2 production during the clotting of the blood in vitro.

Hypothermic cardiopulmonary bypass patients and hypothermic surgical and trauma patients should be rewarmed post-operatively to improve platelet function and to reduce bleeding time and blood loss. In hypothermic bleeding patients who are to receive homologous blood products, both core and peripheral skin temperatures should be rewarmed to normal prior to transfusion. Rewarming is the safest and least expensive method of treating hypothermic patients with non-surgical blood loss, especially in view of the serious potential risks associated with homologous blood products.

# INTRODUCTION

When normal healthy baboons were exposed to systemic and local hypothermia, they exhibited a hypothermia-induced reversible platelet dysfunction: a reduced local skin temperature was associated with a prolonged bleeding time accompanied by a reduction in thromboxane B2 in shed blood collected at the template bleeding time site.[1] Restoration of the baboon's local skin temperature to normal resulted in restoration to normal of both the bleeding time and the level of thromboxane B2 in shed blood.

Studies in patients undergoing hypothermic cardiopulmonary bypass surgery showed a correlation between non-surgical blood loss within the first 4 hours after surgery and the bleeding time measurement made 2 hours after surgery[2]. During the cardiopulmonary bypass period, a reduction in local skin temperature was associated with an increase in bleeding time and a reduction in the level of thromboxane B2 in the shed blood collected at the template bleeding time site. During the 2-hour to 24-hour post-operative period, the bleeding time decreased, the shed blood thromboxane B2 level

increased, the mean platelet volume increased, and the local skin temperature at the site of the bleeding time measurement increased. In the 2-to 24-hour postoperative period during which bleeding time was reduced these patients exhibited a release of large platelets into the circulation and an increase in skin temperature at the template bleeding time site.

The effect of local skin temperature in hypothermic cardiopulmonary bypass surgery patients and its effect on the bleeding time were studied. One of the arms of the hypothermic patient was warmed and the other arm cooled.Bleeding times were measured on both arms and the shed blood was collected from the bleeding time incisions and assayed for thromboxane B2 [3]. A 6C reduction in the local skin temperature of one arm resulted in an increase in the bleeding time of 3 minutes and a significant reduction in the shed blood thromboxane B2 level.

We assessed the effects of temperature on the bleeding time in normothermic male and female volunteers at local skin temperatures

ranging from +38C to +20C. The shed blood from the bleeding time incision was collected and assayed for thromboxane B2. In blood that was clotted with and without agitation at temperatures ranging from +38C to +20C thromboxane B2 was measured in the serum obtained from the clotted blood.

Studies were done to assess the effect of aggregating platelets in vitro at +37C and +22C on the magnitude of aggregation and the ability of the platelet to produce thromboxane after stimulation with either a combination of arachidonic acid (AA) and adenosine diphosphate (ADP) or with ristocetin alone were measured.

## MATERIAL AND METHODS

Forty-one healthy volunteers, 19 males and 22 females between the ages of 19 to 29, participated in this study which was approved by the Institutional Review Board at Boston University School of Medicine. Each volunteer signed an informed consent form. These volunteers were taking no medication.

Sixteen volunteers, eight (8) male and eight (8) female were studied at four measured local skin temperatures of +22C, +28C, +32C, and +37C. Ten volunteers five, (5) males and five (5) females were studied at seven measured local skin temperatures of +20C; +23C; +26C; +29C; +32C; +35C; and +38C. To assess three methods to collect the shed blood at the template bleeding time site nine volunteers, (6) females and (3) males were studied at a measured local skin temperature of +32C, and six volunteers (3) males and (3) females were studied at three measured local skin temperatures of +22C, +28c, and +32C. The forearm skin temperature of the volunteers was equilibrated to temperatures between +20C and +38C by one or more of the following methods: a stream of air cooled by dry ice or wet ice, a walk-in 4C

cold room, a hair dryer, and a heating lamp. Local skin temperature was monitored at thirty second intervals by a surface thermometer (Skin Temperature Sensor, Mon-A-Therm, Inc., St Louis, MO) placed within a few millimeters of the bleeding time site. Duplicate vertical bleeding times were measured according to the method of Babson and Babson[4].

Each bleeding time procedure produced two skin incisions. In all forty-one studies one template bleeding time was used for measurement of the bleeding time: the mean bleeding time of the two skin incisions is reported. In sixteen studies the blood collected on the filter paper during the bleeding time procedure was recovered and the total hemoglobin was measured using the cyanmethemoglobin method: the total hemoglobin is reported in milligrams.

In sixteen studies the shed blood from a second template bleeding time was collected to measure thromboxane B2. Blood emerging from the bleeding time was collected at thirty second intervals with a blunt end needle attatched to a 1 ml syringe containing heparin (1000 U/ml) and 40 lambda of ibuprofen (1.9mg/ml) until a volume of 600 lambda was

collected. In the same sixteen studies where the local skin temperature was +37C, +32C, +28C, and +22C peripheral venous blood samples were collected from the antecubital vein of the locally cooled or warmed forearm. These blood samples were collected into tubes coated with heparin (1,000 U/ml; USP) and containing 40 lambda of ibuprofen (1.9 mg/ml), and were kept on wet ice until the blood was centrifuged at 1650 X g (3000 RPM) in a Sorvall GLC-3 centrifuge for 10 minutes; the plasma was removed and frozen and stored at -80C. Thromboxane B2 levels were measured on the thawed samples. Blood samples were also collected and allowed to clot without agitation at room temperature, and the serum was removed and frozen at -80C. Thromboxane B2 level measurements were made on the thawed samples. Blood samples from the same sixteen volunteers were collected for measurement of hemoglobin concentration (gm%), hematocrit (V%), white blood cell count (#/ul), platelet count (#/ul), and the mean platelet volume (MPV $u^3$) using the Coulter Counter JT Instrument. In addition, partial thromboplastin time (PTT/seconds), prothrombin time (PT/seconds), and factors V and VIII clotting proteins (percent of

normal) were measured in vitro at +37C and +22C in blood samples collected in sodium citrate: the ratio was one volume of 3.8% sodium citrate to 9 volumes of blood[5]. Platelet aggregation and the production of thromboxane B2 in platelet rich plasma prepared from sodium citrated blood stimulated with a combination of arachidonic acid (0.05 mg/ml AA) and adenosine diphosphate (0.01 mM ADP), and ristocetin alone (1.25 mg/ml) were measured. The area under the aggregation curve at the five minute time point was measured by a digitizer and reported as digitizer units/5 minutes. Five minutes after stimulation of the platelets with AA and ADP thromboxane B2 production was stopped by the addition of 1 mg/ ml ibuprofen. The sample was centrifuged at 1600 x g for 10 minutes, and the plasma was frozen and stored at -20C until the thromboxane B2 assay was done. The thromboxane B2 per $10^{-5}$ platelet is reported.

In nine studies the shed blood emerging from the bleeding time site was collected for thromboxane B2 measurement using three different methods at a local skin temperature of +32C as follows: (a)

at thirty second intervals blood was collected into heparin (1000 u/ML) and ibuprofen (1.9 MG/ML) until a volume of 600 lambda was collected; (b) all the shed blood was collected from one bleeding time site at thirty second intervals into heparin and ibuprofen; and (c) shed blood was collected at 2-minute intervals for the duration of the bleeding time. In six studies shed blood was collected using these three above mentioned methods at local skin temperatures of +22C, +28C, and +32C. Shed blood was kept on ice until it was centrifuged at 1650 X g (3000 RPM) in a Sorvall GLC-3 centrifuge for 10 minutes, the supernatant was removed and was frozen and stored at -80C until the measurements were made. Thromboxane B2 measurements were done on the thawed samples using the thromboxane B2 ($^{125}I$) RIA Kits (New England Nuclear Corp., Boston, MA). In six studies the thromboxane $B_2$ production rate (pg/ml/second) during the bleeding time measurement was calculated from the thromboxane $B_2$ level in all the shed blood collected at the bleeding time site and the length of the bleeding time in seconds.

In five in vitro studies aggregation and thromboxane B2 production in response to a combination of AA and ADP or ristocetin alone at +37C and +22c was measured. In sixteen in vitro studies peripheral venous blood was collected for clotting time measurements at +22C, +28C, +32C, and +37C. Clotting times were done in 3.5 ml siliconized glass tubes with 1 or 3 ml of blood, or in 7 ml siliconized tubes with 7 ml of blood: the tube was agitated every 30 seconds until a clot was formed. Matched blood samples were allowed to clot non agitated at the same temperature in the same size tubes for the time required for the blood to clot in the tubes that were agitated. The serum was separated from all the clotted blood samples by centrifugation and was frozen at -80C. Thromboxane B2 measurements were done on the thawed samples. The thromboxane B2 production rate (pg/ml/second) during the clotting of the blood was calculated from the serum thromboxane level and the length of the clotting time in seconds in the agitated samples.

Three studies were done to assess thromboxane B2 levels at 30 second intervals during the clotting of blood at +37C, +32C, +28C, and+22C .

One ml samples were collected into 3.0 ml siliconized glass tubes and each blood sample was agitated every 30 seconds until the addition of ibuprofen to halt the production of thromboxane. Five one ml aliquots were studied at each temperature and ibuprofen added to each tube successively. The samples were centrifuged and the serum frozen at -80C until assayed for thromboxane B2 level.

The actual value of the bleeding time, serum, plasma, and shed blood levels of thromboxane B2, and the total hemoglobin on the filter paper were reported as well as the natural logarithm of each value. The hematocrit, hemoglobin concentration, red blood cell count, platelet count, white blood cell count, MPV, the bleeding time, serum, plasma and shed blood thromboxane B2 level, and total hemoglobin on the filter paper at each temperature were analyzed with a one-way analysis of variance (ANOVA). The paired Students t-test was utilized for comparison of the sample means when the ANOVA was significant ($p<0.05$)[6]. Correlations were done by linear regression analysis. Analyses were done using a statistical software package

(PRODAS, Conceptual Software, Inc., Houston, TX) in an IBM personal computer.

## RESULTS

Tables 1 and 2 report measurements in peripheral blood samples collected from the antecubital vein of the locally cooled or warmed forearm for 16 of the 41 healthy volunteers in the study. Hematological measurements made at local skin temperatures of +37C; +32C; +28C; +22C showed no significant changes in Hct, Hgb concentration, RBC count, WBC count, platelet count, or MPV (Table 1). Partial thromboplastin time (PTT), prothrombin time (PT), factor V, and factor VIII assays were measured at +22C and +37C. The PTT and PT were significantly prolonged and the factor V significantly reduced at +22C compared to +37C (Table 2).

Table 3A and Figures 1 to 8 report bleeding times and the thromboxane B2 levels in shed blood collected at the template bleeding time site and in the heparin-ibuprofen plasma and serum samples from venous blood obtained from the antecubital vein of the locally cooled or warmed forearm. Bleeding times measured at local skin temperatures of +37C, +32C, +28C, and +22C showed a significant increase at +22C (22.5 $\pm$ 7.5 minutes) compared to +37C (5.8 $\pm$ 1.3

minutes). In the shed blood, the thromboxane B2 level was significantly decreased at +22C (240 ± 178 pg/0.1 ml) compared to the level at +37C (3034 ± 1555 pg/0.1 ml). There were no significant differences in the plasma thromboxane B2 levels throughout the 22C to 37C range. The serum thromboxane B2 level did show a significant decrease from 2286 ± 1697 pg/0.1 ml at +37C to 668 ± 717 pg/0.1 at +22C. Table 3B and Figures 9 to 16 report the natural logarithm of bleeding time and thromboxane B2 level in the shed blood collected at the template bleeding time site; in the heparin-ibuprofen plasma; and in the serum from venous blood obtained from the antecubital vein of the locally cooled and warmed forearm maintained at +37C, +32C, +28C, and +22C.

Tables 4A, 4B, 5A, 5B and Figures 17 to 32 report thromboxane $B_2$ levels in shed blood collected from the bleeding time site using three different methods at local skin temperatures of +32C, +28C, and +22C. A volume of 0.6 ml of shed blood was collected; all the shed blood from one bleeding time was collected; and the shed blood was collected at 2 minute intervals throughout the bleeding time

measurement. The highest thromboxane B2 levels were seen in the samples collected during the last 2 minute collection period of the bleeding time measurement at temperatures of +32C and +28C (Tables 4A, 4B, 5A, and 5B). Reduction in the local skin temperature to 28C and 22C increased the bleeding time and reduced the thromboxane B2 level in shed blood collected from the bleeding time site (Tables 4A, 4B, 5A, and 5B, Figures 17 to 32).

Tables 6 to 13; 14A, 14B, 15A, 15B, Figures 33 to 44 report the clotting times and the serum thromboxane B2 levels obtained from blood clotted at +37C, +32C, +28C, and +22C with and without agitation. Agitation of the blood during clotting produced significantly higher serum thromboxane $B_2$ levels at +32C and +37C (Tables 14A AND 14B).

Table 16 and Figures 45, 46, 47, and 48 report the rate of thromboxane production and the natural logarithm of the rate of thromboxane B2 production and in shed blood collected from the bleeding site in a forearm where the local skin temperature was maintained at +22C, +28C, +32C, and +37C, and in blood clotted with

agitation at +22C, +28C, +32C, and +37C. For each 1C decrease in temperature there was a 15% decrease in the rate of thromboxane B2 production in both the shed blood and the agitated clotted blood.

Tables 17A and 17B, Figures 49 and 50 report the bleeding times and total hemoglobin levels collected from the bleeding time on the filter paper at local skin temperatures of +38C, +35C, +32C, 29C, 26C, 23C, and +20C. Bleeding time was significantly increased at +20C (22 $\pm$ 5.0 minutes) compared to +38C (5.3 $\pm$ 1.5 minutes). A significant correlation ($r=0.352, p<0.001$, $n=94$) was observed between bleeding time and the total hemoglobin on the filter paper (Table 17A and 17B, Figure 49 and 50).

Tables 18A, 18B, 19A, and 19B report the aggregation patterns and thromboxane B2 levels in the platelet-rich plasma obtained from sodium citrate venous blood collected from the locally warmed and cooled forearm at temperatures of +37C, +32C, +28C, and +22C. The platelet-rich plasma was stimulated at +37C with either a combination of arachidonic acid (0.05 mg/ml AA) and adenosine diphosphate (0.01 mM ADP) or with 1.25 mg/ml or ristocetin alone. Comparison of 5 minute

aggregation patterns showed no significant differences whether the stimulus was a combination of AA and ADP or ristocetin alone (Tables 18A and 18B). Thromboxane B2 production during the aggregation was halted after five minutes of aggregation with the addition of 1 mg/ml ibuprofen. Table 19A and 19B report that thromboxane $B_2$ production was significantly greater following stimulation of the platelets with a combination of AA and ADP than with ristocetin alone. The aggregation patterns in response to a combination of AA and ADP and to ristocetin alone were similar although the thromboxane $B_2$ production by the platelets was significantly greater in response to AA and ADP than to ristocetin alone.

Tables 20A and 20B report platelet aggregation and the platelet production of thromboxane B2 in vitro at +22C and +37C following stimulation with AA and ADP and to ristocetin alone . Both the platelet aggregation and the platelet production of thromboxane B2 were better at +37C than at +22C; but these differences were not statistically significant.

Table 21 reports measured bleeding times in 10 normothermic volunteers at the 7 skin temperatures.The bleeding times were corrected to a skin temperature of 35C using the empirically derived factor: (T - 35C)/20C + 1, where T was the measured skin temperature when T was less than 35C.

Figure 51 reports the mean and standard deviation of the bleeding times in the 10 volunteers in whom bleeding time was measured at each of the 7 temperatures and corrected by the above factor.

## DISCUSSION

Many factors are known to influence the bleeding time measurement, e.g., platelet count, mean platelet volume, platelet mass, von Willebrand's factor, factor VIII clotting protein, capillary integrity, vessel reactivity, drugs which affect platelet function, and mechanical events related to local perfusion pressure, resistance, and blood flow[7-16].

Previous studies in baboons and in patients undergoing extracorporeal bypass surgery have shown increased bleeding times associated with reduced local skin temperatures and reduced levels of thromboxane B2, the stable metabolite of thromboxane B2, in shed blood collected at the template bleeding time site[1-3]. The shed blood level of 6-keto PGF1a, the stable metabolite of prostacyclin, did not appear to affect the bleeding time[1-3]. In the present study involving healthy male and female volunteers, a reduced local skin temperature was found to have a definite effect on bleeding time: when the local skin temperature was reduced from 35C to 22C, the bleeding time increased 3 to 4 times[17]. However when the local skin temperature was

increased from 35 to 38C, no significant effect on the bleeding time was observed.

The thromboxane B2 level in the shed blood collected from the template bleeding time site was influenced by the local skin temperature; decrease in local skin temperature significantly reduced the shed blood level of thromboxane B2 and significantly increased the bleeding time. The thromboxane B2 level in the serum from the blood collected from the warmed or cooled forearm and allowed to clot at room temperature without agitation was also influenced by local skin temperature. Blood clotted without agitation at +22C, +28C, +32C, and +37C showed a non significant increase in the serum thromboxane $B_2$ level as the temperature increased. However, blood clotted with agitation at +22C, +28C, +32C, and +37C showed a significant increase in the serum thromboxane $B_2$ level as the temperature increased. The serum thromboxane $B_2$ levels in blood clotted with agitation at +32C and +37C were significantly higher than the serum thromboxane $B_2$ levels in blood clotted without agitaion at +32C and +37C. These data show that the serum thromboxane $B_2$ level is influenced by both

temperature and agitation of the blood during the clotting time. The data show that rate of thromboxane B2 production decreased by 15% for each 1C decrease in temperature in both the shed blood and the agitated clotted blood.

Our data in healthy volunteers show that temperature plays an important role in bleeding time and clotting time measurements. Our results further underscore the necessity of measuring skin temperature when measuring bleeding time and of correcting the measured bleeding time for the measured skin temperature using the empirically derived factor: T - 35C/20C+1 where T is the measured skin temperature and when T is less than 35C.[17] Only by this method is it possible to learn whether the bleeding time measurement has been influenced by the individual's platelets, clotting proteins, capillary integrity, vessel reactivity and other mechanical factors and by drugs, anesthesia, and transfused blood products.

In patients undergoing hypothermic cardiopulmonary bypass surgery, non-surgical blood loss during the 4-hours post-operative period was found to correlate with the bleeding time 2 hours post-op[2].

In the healthy volunteers the bleeding time correlated with the total hemoglobin recovered from the filter paper used to measure the bleeding time. Unlike reports to the contrary,[18,19] our data in previous reports [2,3] and in this study show that bleeding time correlates to non-surgical blood loss. Our data also support that a reduction in non-surgical blood loss is best achieved by cooling the periphery of the bleeding site and warming the bleeding time site[20,21].

Prevention of a bleeding diathesis generally associated with resuscitation of hypothermic patients in hemorrhagic shock, can be achieved by rewarming the patients to restore both core and peripheral temperature to normal. This would ensure optimum function of the patient's platelets and clotting proteins. Rewarming is critical to improved platelet function and reductions in both bleeding time and blood loss in hypothermic cardiopulmonary bypass patients and in hypothermic surgical and trauma patients. In view of the serious potential risks associated with homologous blood products, rewarming

is the safest treatment for hypothermic patients with non-surgical blood loss, as well as the least expensive.

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TABLE 1

**THE HEMATOLOGIC MEASUREMENTS IN PERIPHERAL BLOOD COLLECTED FROM THE ANTECUBITAL VEIN OBTAINED FROM NORMAL MALE AND FEMALE VOLUNTEERS SUBJECTED TO LOCAL COOLING AND WARMING OF THE FOREARM TO ACHIEVE LOCAL SKIN TEMPERATURES OF +37, +32, +28, AND +22C**

| Temp | PLT Count ($x10^3$/ul) | HCT (V%) | HGB (GM%) | WBC Count ($x10^3$/ul) | RBC (x106/ul) | MPV ($u^3$) |
|---|---|---|---|---|---|---|
| **37C** | | | | | | |
| Mean: | 254 | 37 | 12 | 5.1 | 3.9 | 7.6 |
| SD: | 66 | 10 | 3 | 2 | 1.1 | 2.1 |
| n: | 15 | 16 | 16 | 16 | 16 | 16 |
| **32C** | | | | | | |
| Mean: | 264 | 38 | 13 | 5.1 | 4.0 | 7.5 |
| SD: | 70 | 10 | 4 | 2 | 1.1 | 2.1 |
| n: | 15 | 16 | 16 | 16 | 16 | 16 |
| **28C** | | | | | | |
| Mean: | 255 | 37 | 12 | 5.3 | 3.9 | 7.9 |
| SD: | 69 | 10 | 3 | 2 | 1.1 | 2.2 |
| n: | 15 | 16 | 16 | 16 | 16 | 16 |
| **22C** | | | | | | |
| Mean: | 262 | 36 | 12 | 6.0 | 3.9 | 7.7 |
| SD: | 69 | 10 | 3 | 2 | 1.1 | 2.2 |
| n: | 15 | 16 | 16 | 16 | 16 | 16 |
| 1 Way ANOVA: | ns | ns | ns | ns | ns | ns |

TABLE 2

**THE MEASUREMENT OF THE PTT, PT, FACTOR V AND FACTOR VIII CLOTTING PROTEINS AT 22C AND 37C IN SODIUM CITRATE PLASMA OBTAINED FROM NORMAL VOLUNTEERS AT A LOCAL SKIN TEMPERATURE OF +32C**

| n=4 | 37C | Paired t TEST Between 37C & 22C | 22C |
|---|---|---|---|
| PTT (Sec) | | | |
| Mean: | 46 | | 95 |
| SD: | 2 | <.001 | 52 |
| PT (Sec) | | | |
| Mean: | 17 | | 40 |
| SD: | 1.5 | <.001 | 2 |
| Factor V (% of normal) | | | |
| Mean: | 69 | | 29 |
| SD: | 44 | <.05 | 21 |
| Factor VIII (% of normal) | | | |
| Mean: | 54 | NS | 44 |
| SD: | 12 | | 4 |

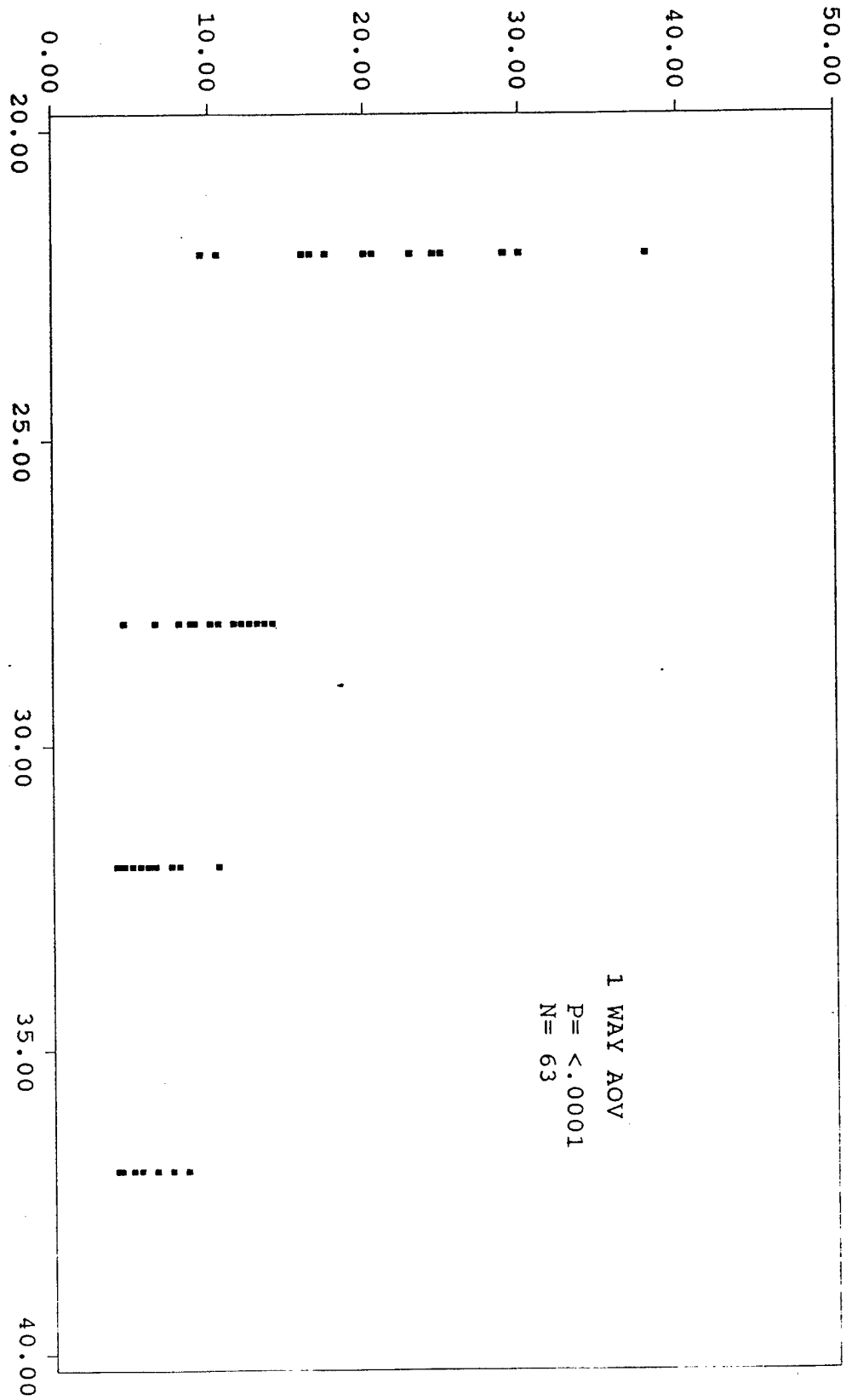
TABLE 3A

**THE BLEEDING TIME AND THE LEVELS OF THROMBOXANE $B_2$ IN THE SHED BLOOD, THE VENOUS HEPARIN-IBUPROFEN PLASMA, AND VENOUS SERUM OBTAINED FROM NORMAL VOLUNTEERS SUBJECTED TO LOCAL COOLING AND WARMING OF THE FOREARM TO ACHIEVE LOCAL SKIN TEMPERATURES OF +37C, +32C, +28C, AND +22C**

| Temp | Bleeding Time (min) | Shed Blood TxB2 (pg/ 0.1 ml) in 0.6 ml of shed blood | Venous Heparin-Ibuprofen Plasma TxB2 (pg/ 0.1 ml) | Venous Serum TxB2 (pg/ 0.1 ml) |
|---|---|---|---|---|
| **37C** | | | | |
| Mean: | 5.8 | 3034 | 31 | 2286 |
| SD: | 1.3 | 1555 | 15 | 1697 |
| n: | 16 | 15 | 16 | 16 |
| **32C** | | | | |
| Mean: | 6.3 | 2010 | 31 | 2174 |
| SD: | 2 | 1357 | 16 | 1154 |
| n: | 16 | 15 | 16 | 16 |
| **28C** | | | | |
| Mean: | 10.3 | 422 | 33 | 1678 |
| SD: | 3 | 213 | 10 | 1008 |
| n: | 16 | 15 | 16 | 16 |
| **22C** | | | | |
| Mean: | 22.5 | 240 | 28 | 668 |
| SD: | 7.5 | 178 | 9 | 717 |
| n: | 16 | 15 | 15 | 16 |
| 1-Way ANOVA: | <.001 | <.001 | ns | <.05 |

FIGURE 1

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE BLEEDING TIME IN NORMAL VOLUNTEERS



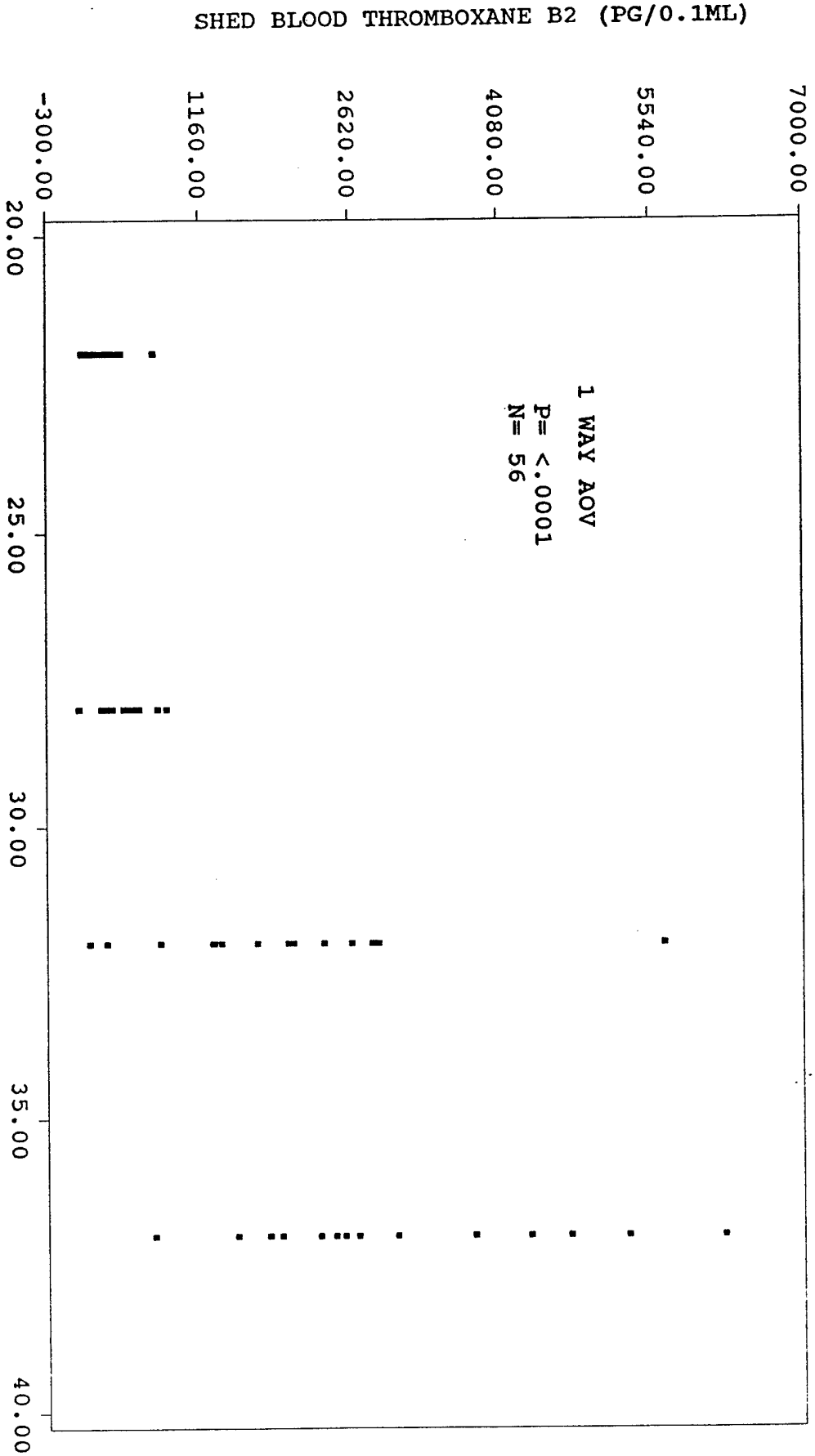



FIGURE 2

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE SHED BLOOD THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS

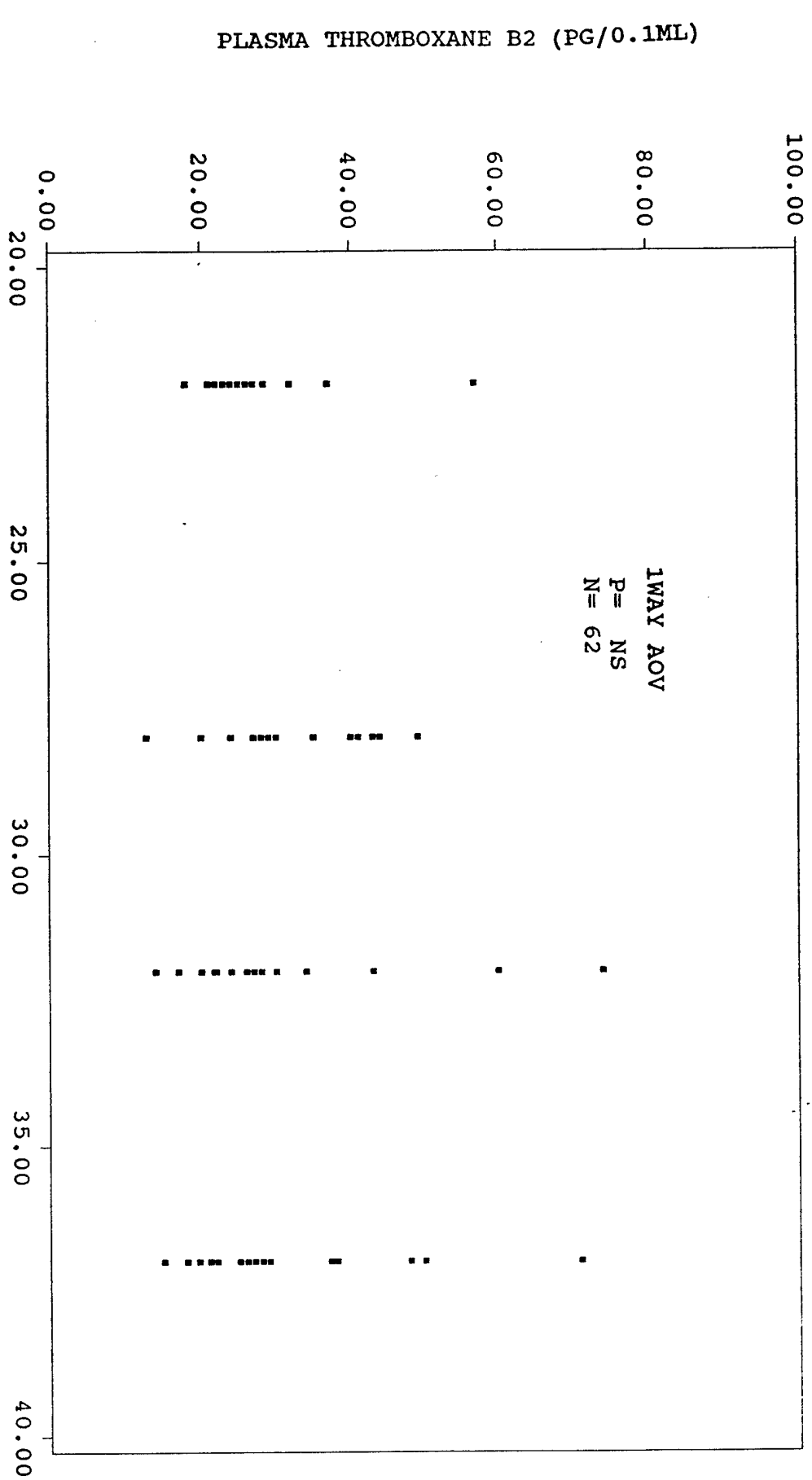



FIGURE 3

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE PLASMA THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS

FIGURE 4

THE RELATIONSHIP BETWEEN THE SKIN TEMPERATURE AND THE SERUM THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS

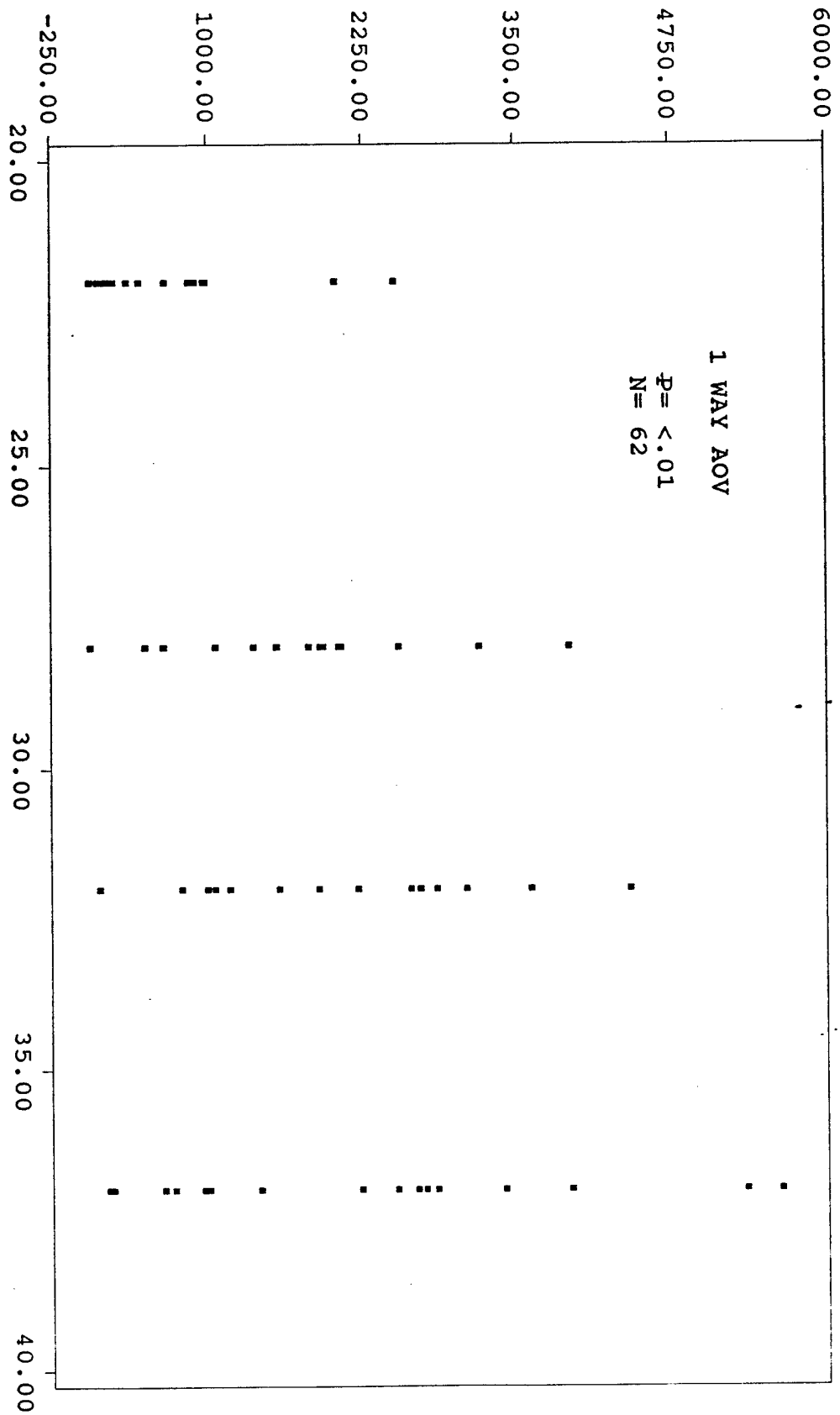


FIGURE 5

DISTRIBUTION OF THE BLEEDING TIME IN MINUTES IN NORMAL VOLUNTEERS

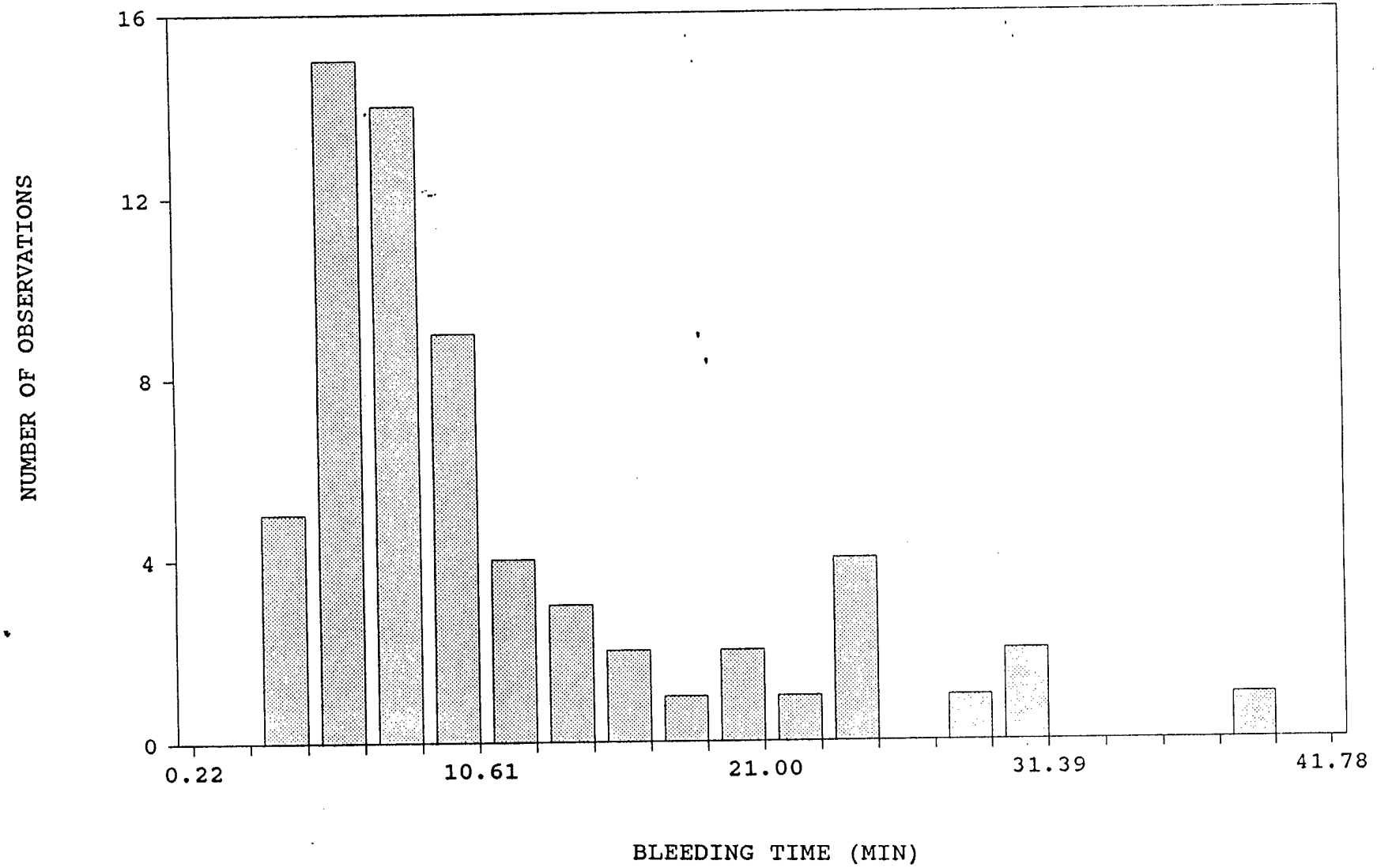
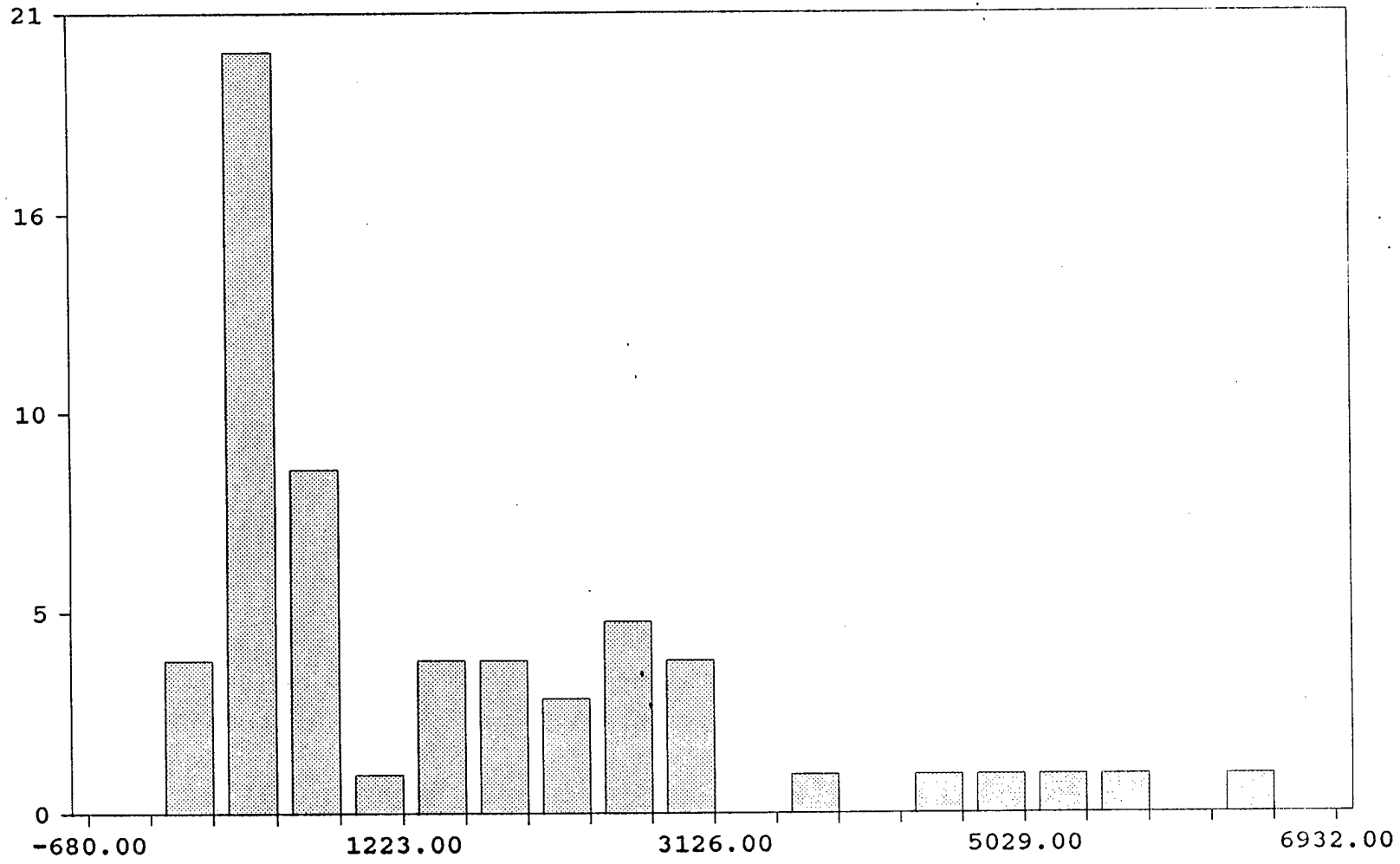


FIGURE 6

DISTRIBUTION OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS



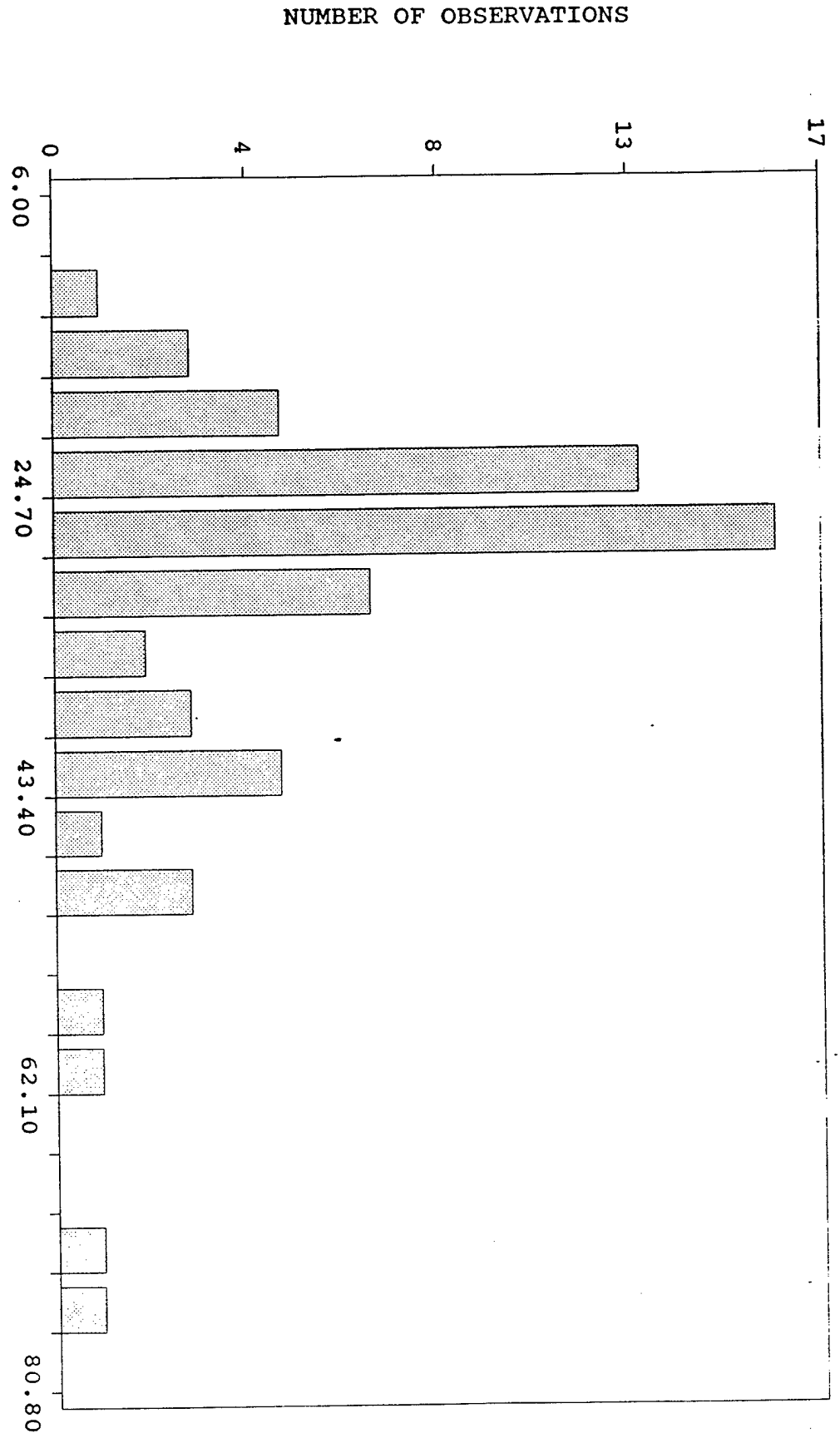



FIGURE 7

DISTRIBUTION OF THE PLASMA THROMBOXANE $B_2$ LEVELS IN NORMAL VOLUNTEERS

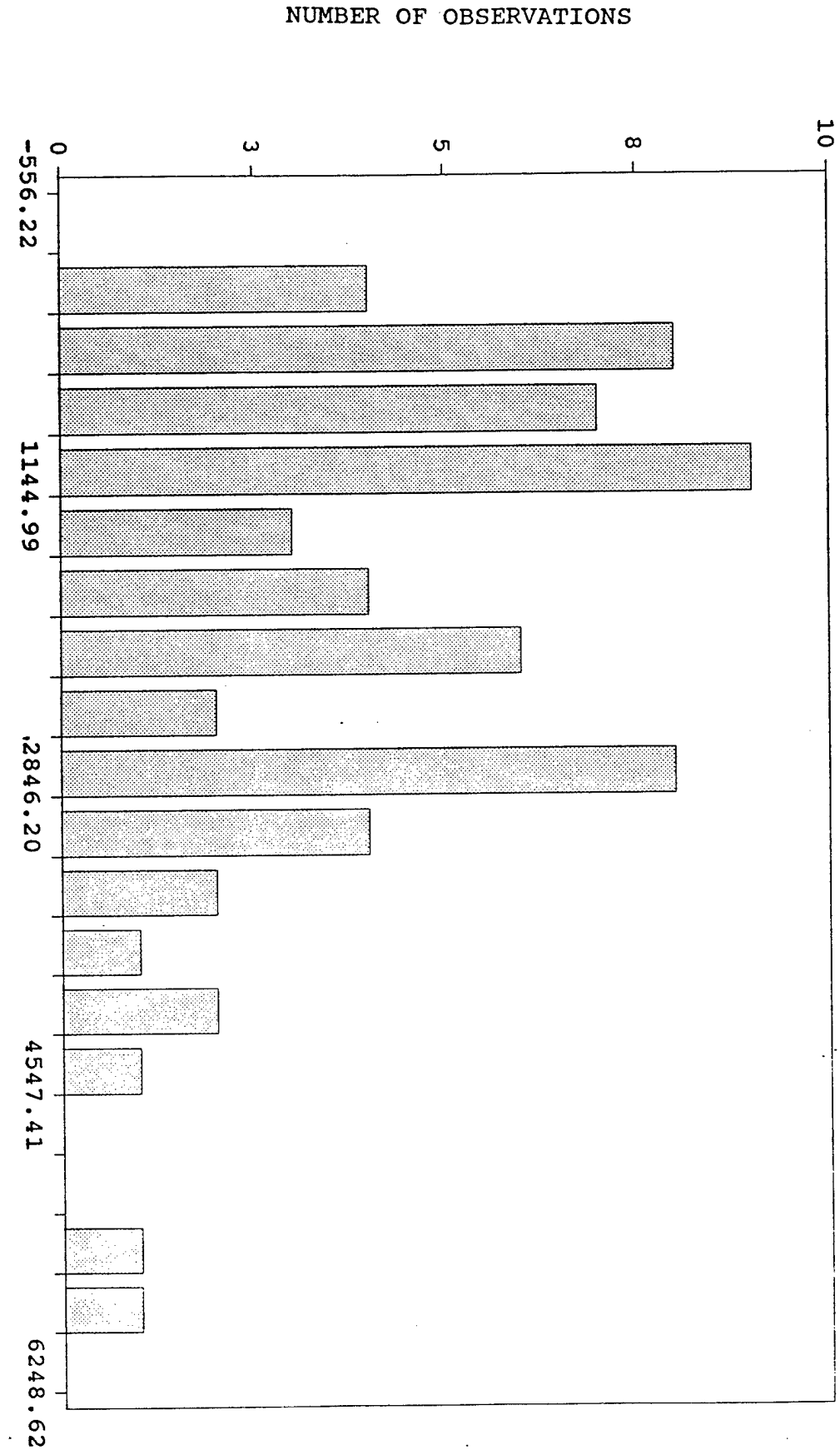



FIGURE 8

DISTRIBUTION OF THE SERUM THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS

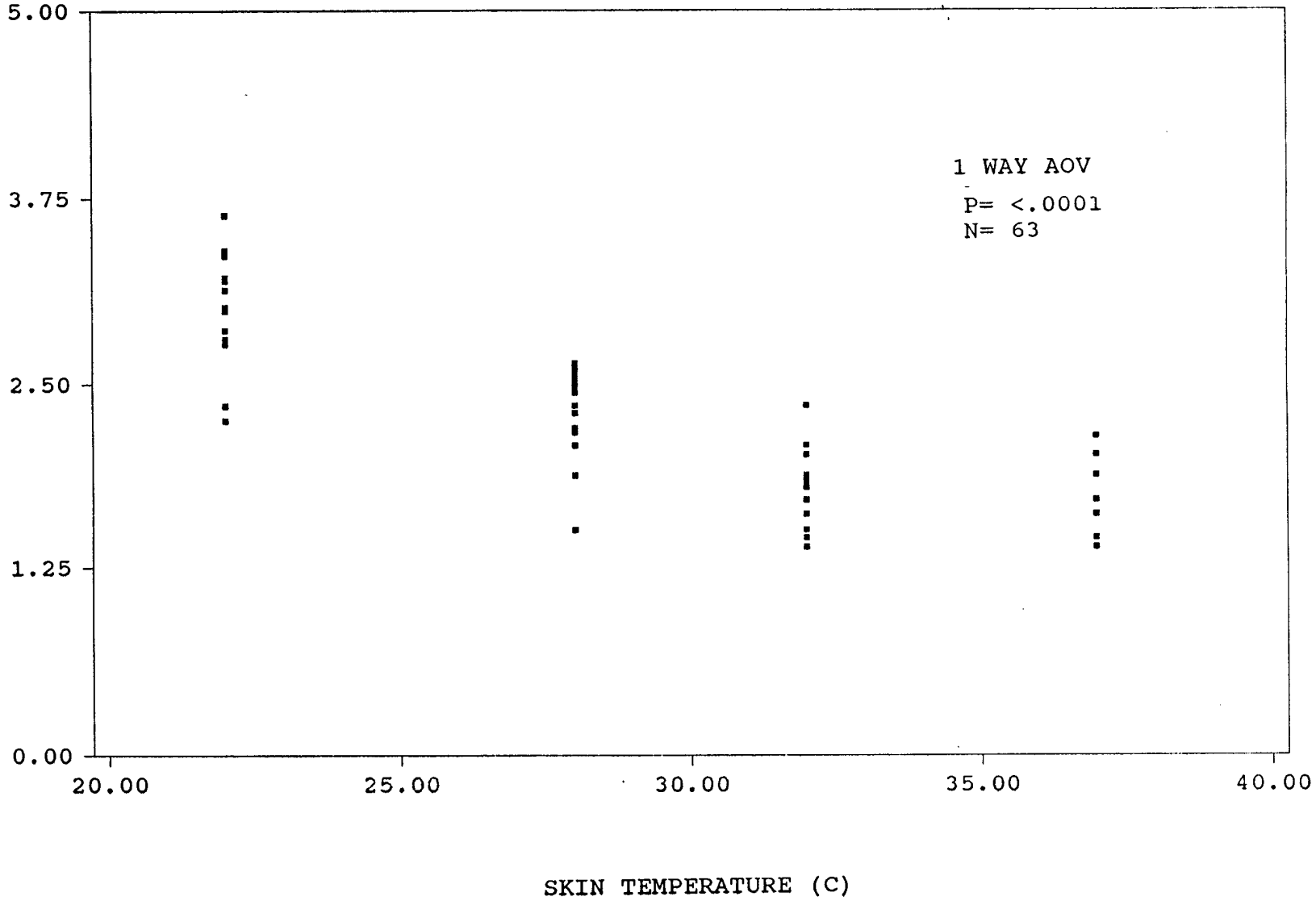
TABLE 3B

**THE NATURAL LOGARITHM OF THE BLEEDING TIME AND THE LEVELS OF THROMBOXANE $B_2$ IN THE SHED BLOOD, THE VENOUS HEPARIN-IBUPROFEN PLASMA, AND VENOUS SERUM OBTAINED FROM NORMAL VOLUNTEERS SUBJECTED TO LOCAL COOLING AND WARMING OF THE FOREARM TO ACHIEVE LOCAL SKIN TEMPERATURES OF +37C, +32C, +28C, AND +22C**

| Temp | Bleeding Time (min) | Shed Blood TxB2 (pg/ 0.1 ml) in 0.6 ml of shed blood | Venous Heparin-Ibuprofen Plasma TxB2 (pg/ 0.1 ml) | Venous Serum TxB2 (pg/ 0.1 ml) |
|---|---|---|---|---|
| **37C** | | | | |
| Mean: | 1.73 | 7.8 | 3.28 | 7.36 |
| SD: | .2 | .5 | .4 | 1.0 |
| n: | 16 | 15 | 16 | 16 |
| **32C** | | | | |
| Mean: | 1.81 | 7.29 | 3.32 | 7.46 |
| SD: | .3 | 1.0 | .4 | .8 |
| n: | 16 | 15 | 16 | 16 |
| **28C** | | | | |
| Mean: | 2.3 | 5.80 | 3.44 | 7.14 |
| SD: | .3 | .9 | .3 | .9 |
| n: | 16 | 15 | 16 | 16 |
| **22C** | | | | |
| Mean: | 3.05 | 5.21 | 3.28 | 6.07 |
| SD: | .4 | .8 | .3 | 1.1 |
| n: | 16 | 15 | 15 | 15 |
| 1 Way ANOVA: | <.001 | <.001 | ns | <.001 |

FIGURE 9

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE BLEEDING TIME AND THE SKIN TEMPERATURE



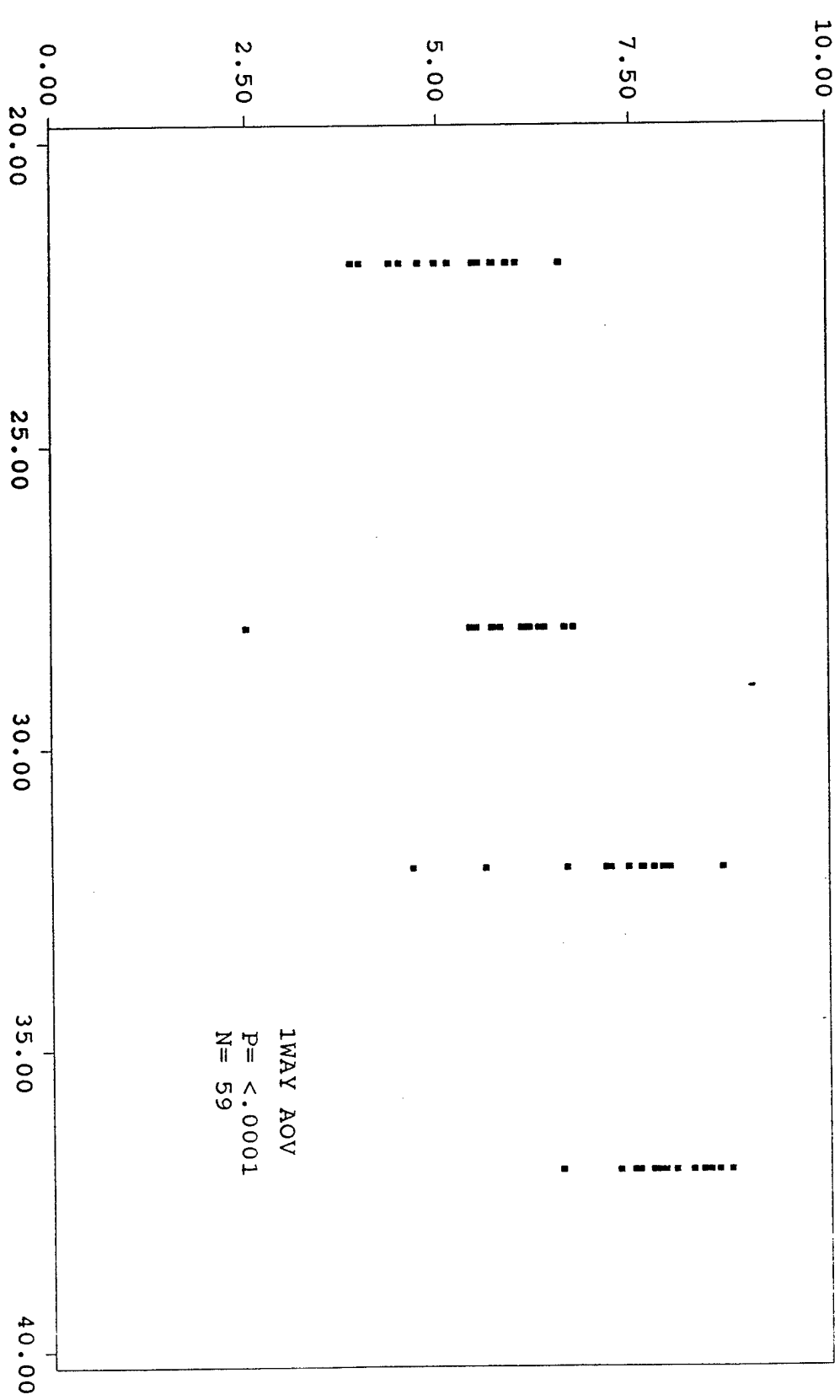



FIGURE 10

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL AND THE SKIN TEMPERATURE

FIGURE 11

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE PLASMA THROMBOXANE $B_2$ LEVEL AND THE SKIN TEMPERATURE

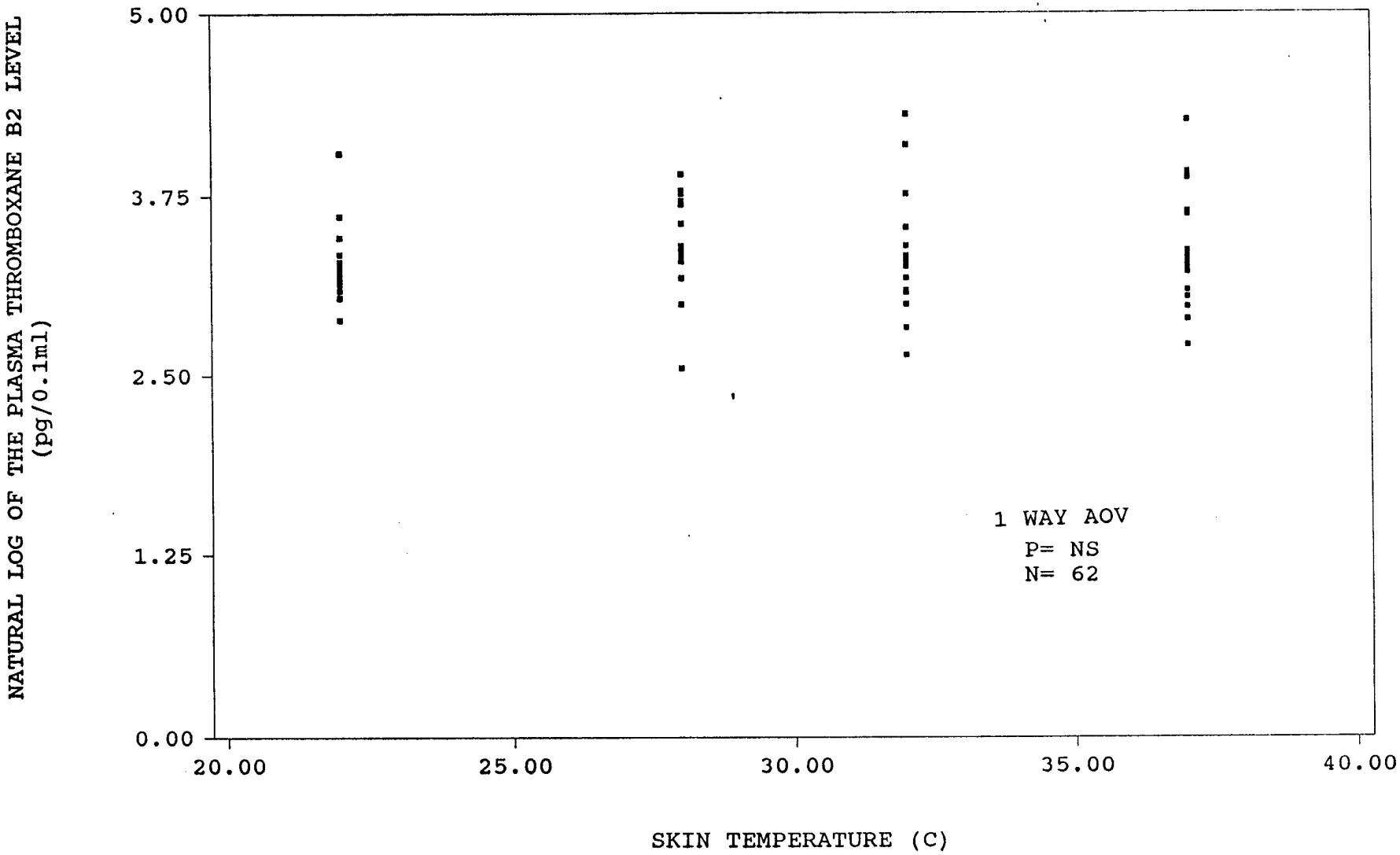


FIGURE 12

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE $B_2$ LEVEL AND THE SKIN TEMPERATURE

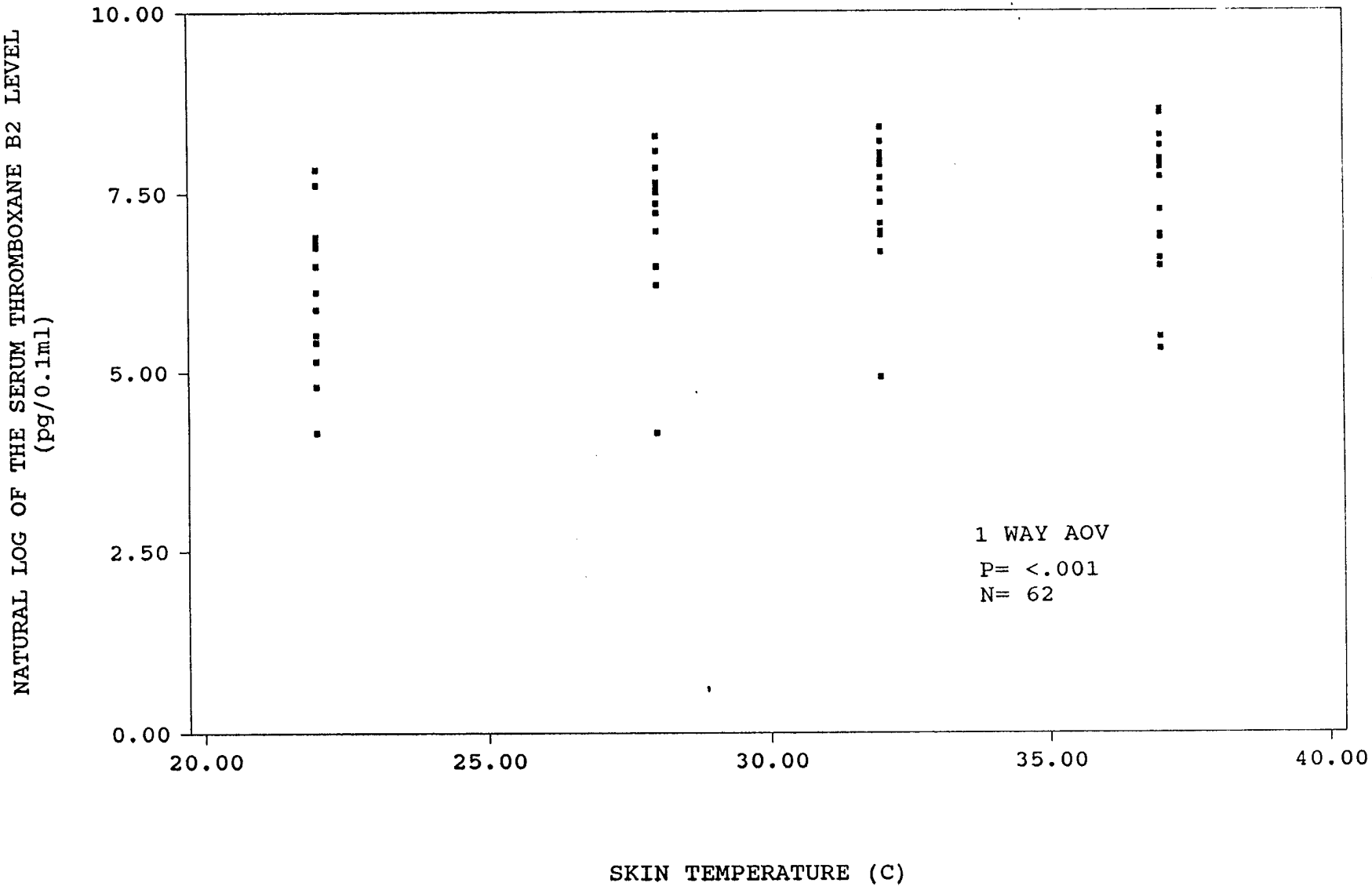


FIGURE 13

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE BLEEDING TIME IN NORMAL VOLUNTEERS

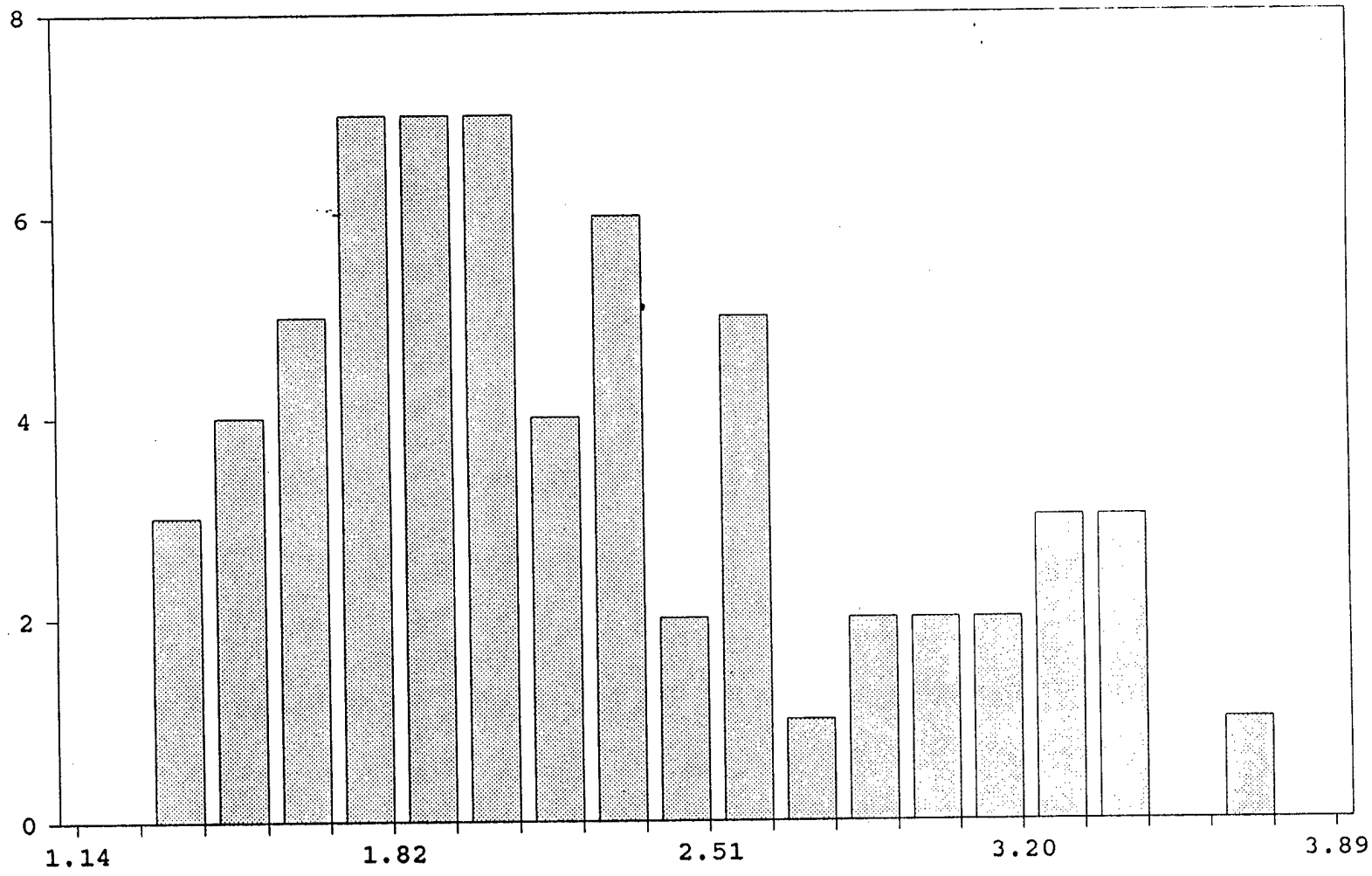
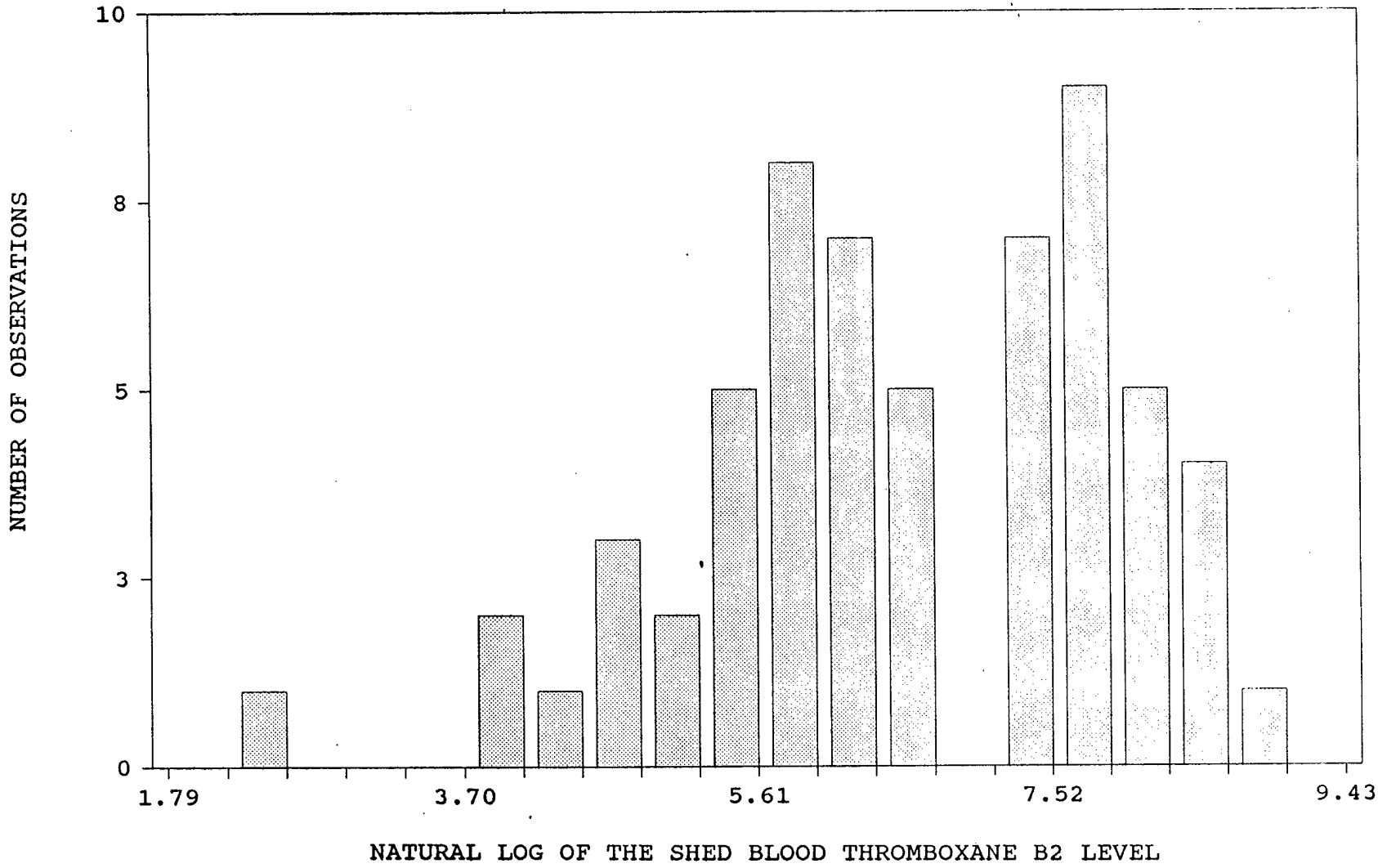


FIGURE 14

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS



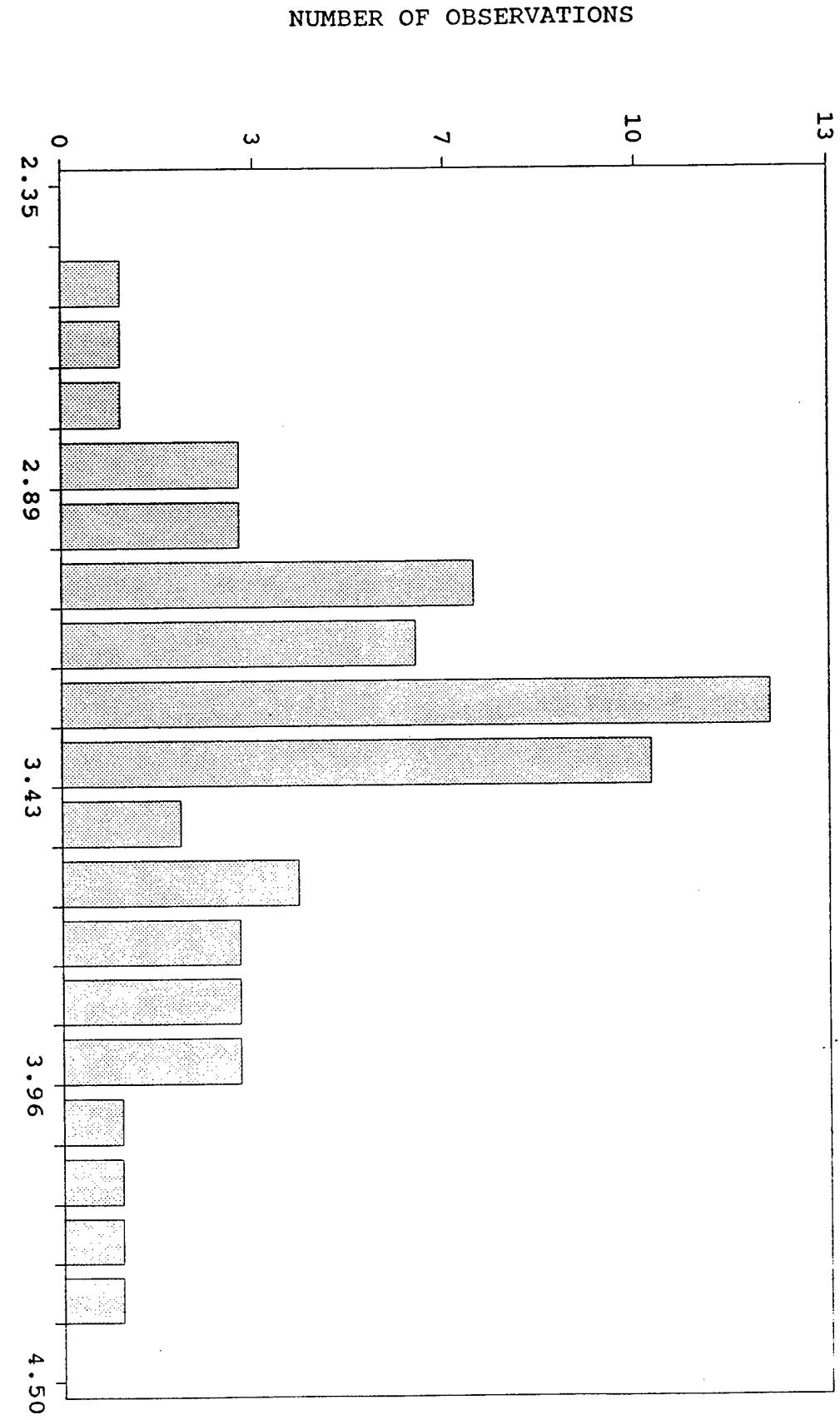



FIGURE 15

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE PLASMA THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS

FIGURE 16

DISTRIBUTION OF THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE $B_2$ LEVEL IN NORMAL VOLUNTEERS

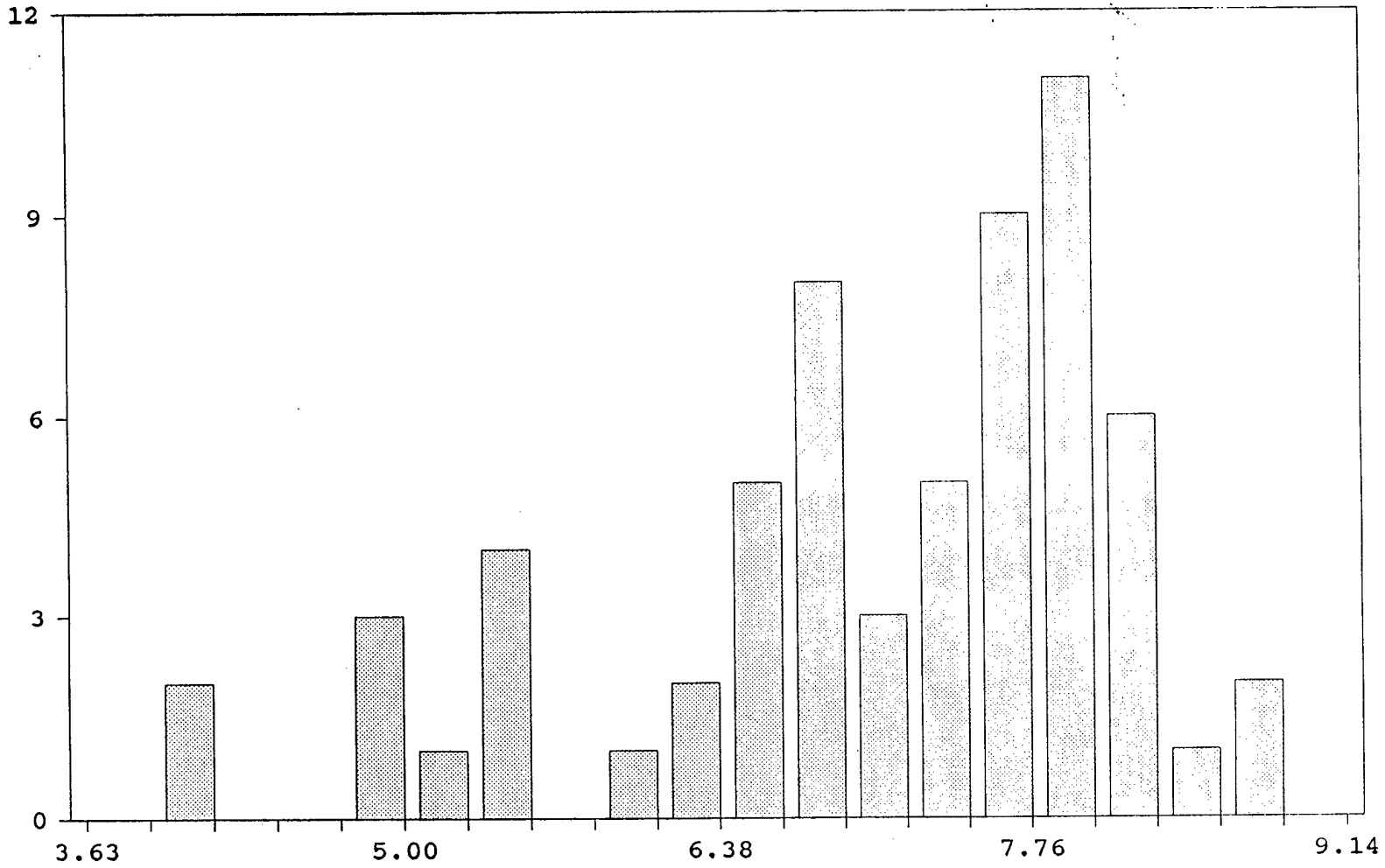


TABLE 4A

**THE THROMBOXANE $B_2$ LEVEL IN A 0.6 ML VOLUME OF SHED BLOOD; IN ALL THE SHED BLOOD FROM ONE BLEEDING SITE AND IN TWO MINUTE COLLECTION INTERVALS AT ONE BLEEDING TIME SITE AND THE LEVEL IN THE FIRST 2 MINUTE INTERVAL AND THE LAST 2 MINUTE INTERVAL REPORTED TOGETHER WITH THE BLEEDING TIME MEASURED AT THE LOCAL SKIN TEMPERATURE OF +32C**

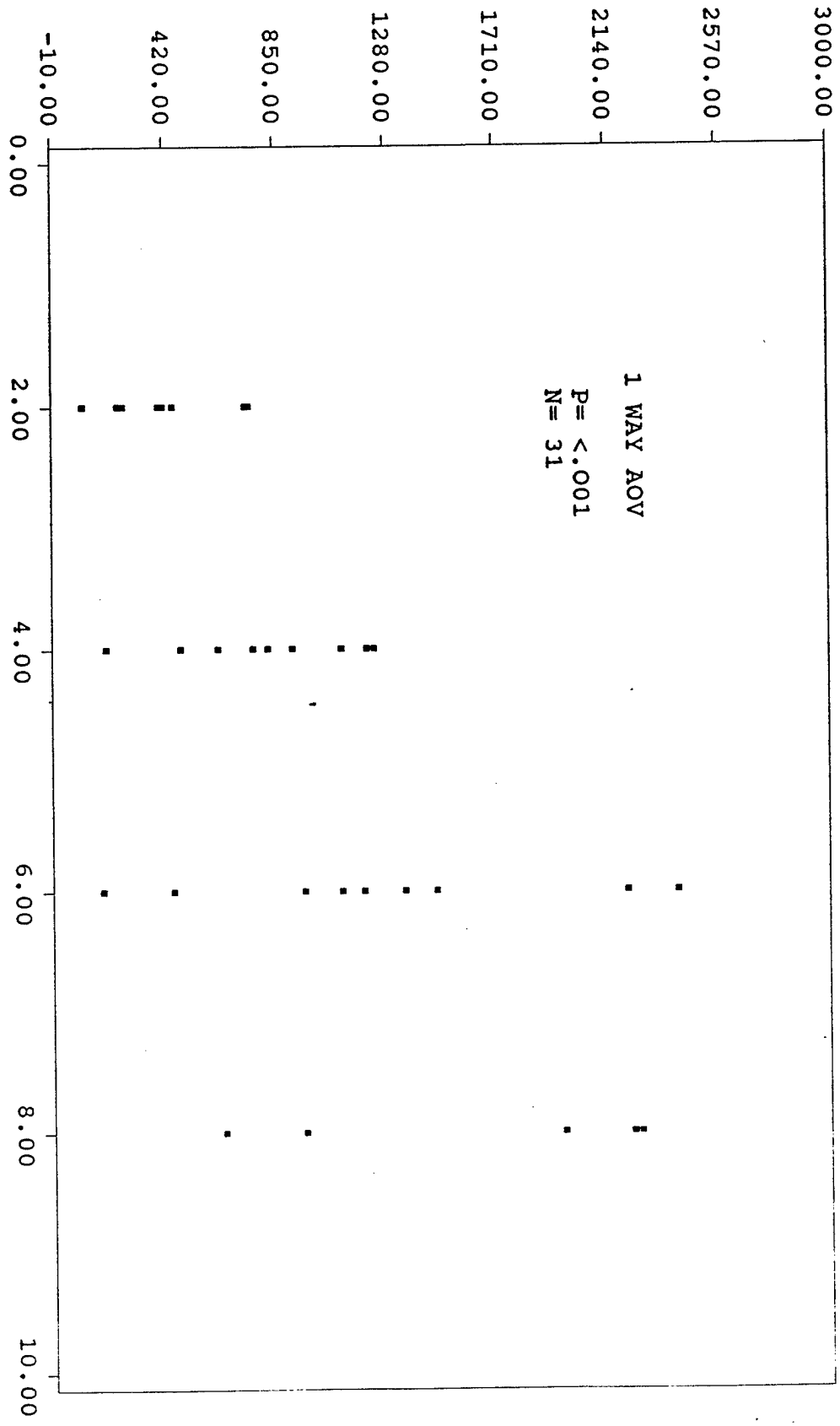
| | Collection to 0.6 ml volume of shed blood pg/0.1 ml | Collection of all the shed blood from 1 site pg/0.1 ml | 2 Minute Collection intervals from 1 site Intervals First 2 min pg/0.1 ml | Last 2 min pg/0.1 ml | Mean BT (min) at 32C |
|---|---|---|---|---|---|
| Mean: | 632 | 1345 | 405 | 1534 | 8.0 |
| SD: | 318 | 745 | 220 | 747 | 3.5 |
| Range: | 278-1134 | 425-2588 | 110-751 | 570-2415 | 6.5-14 |
| n: | 9 | 9 | 9 | 9 | 9 |

Paired T test between

the first two minutes and the last two minute sample <.05

FIGURE 17

THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED DURING THE BLEEDING TIME AT TWO MINUTE INTERVALS



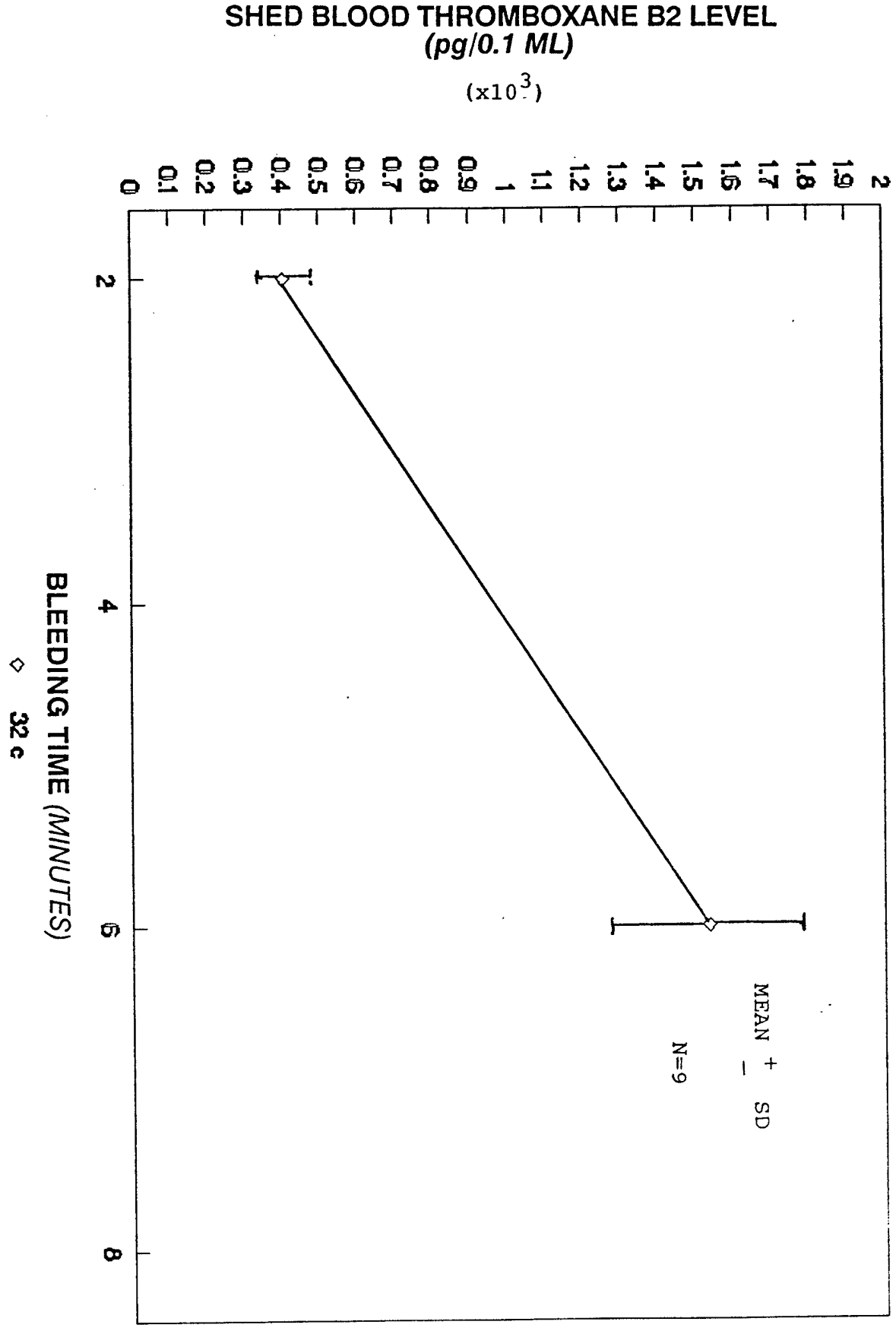



FIGURE 18

THE THROMBOXANE $B_2$ LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

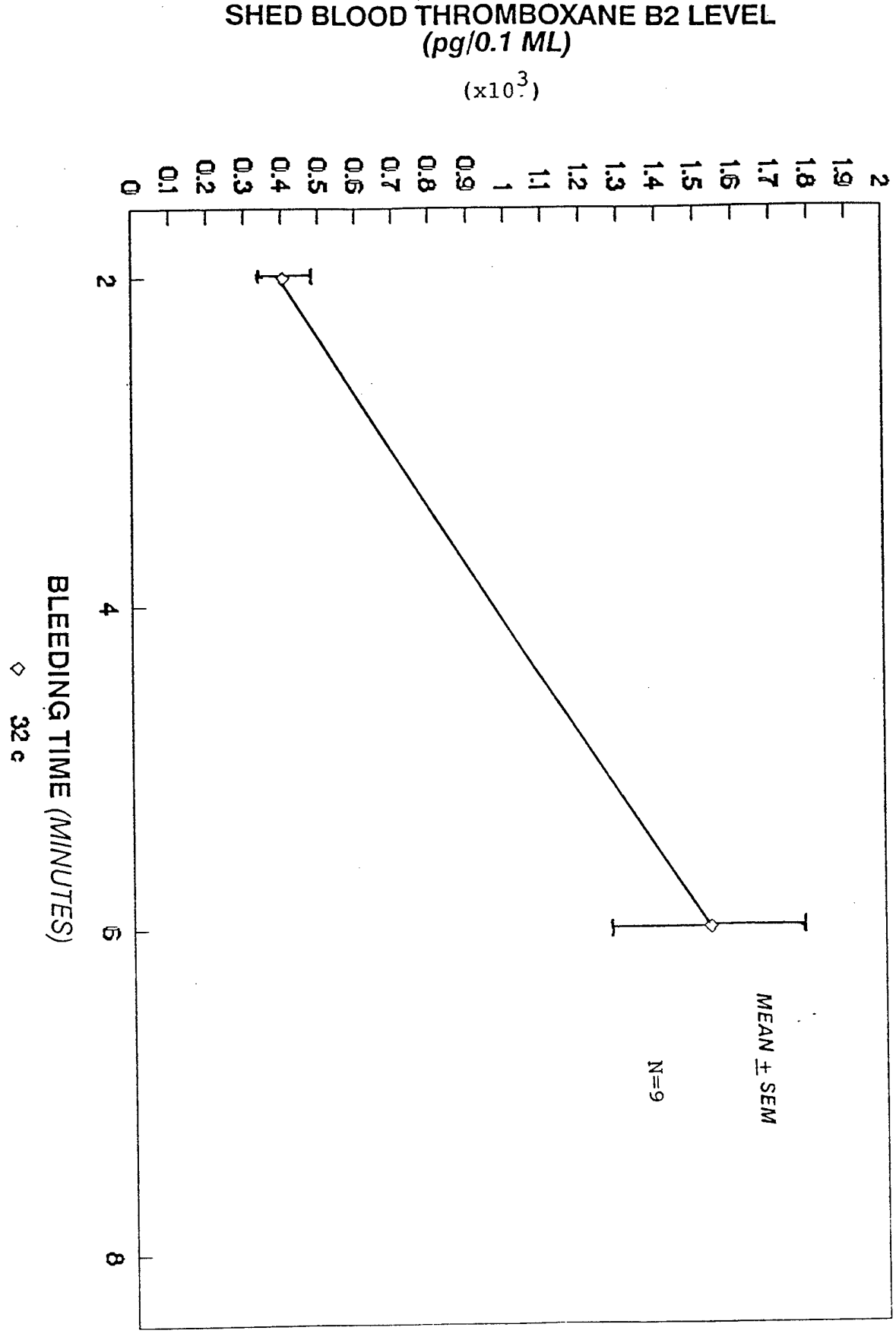



FIGURE 19

THE THROMBOXANE $B_2$ LEVEL IN THE SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

TABLE 4B

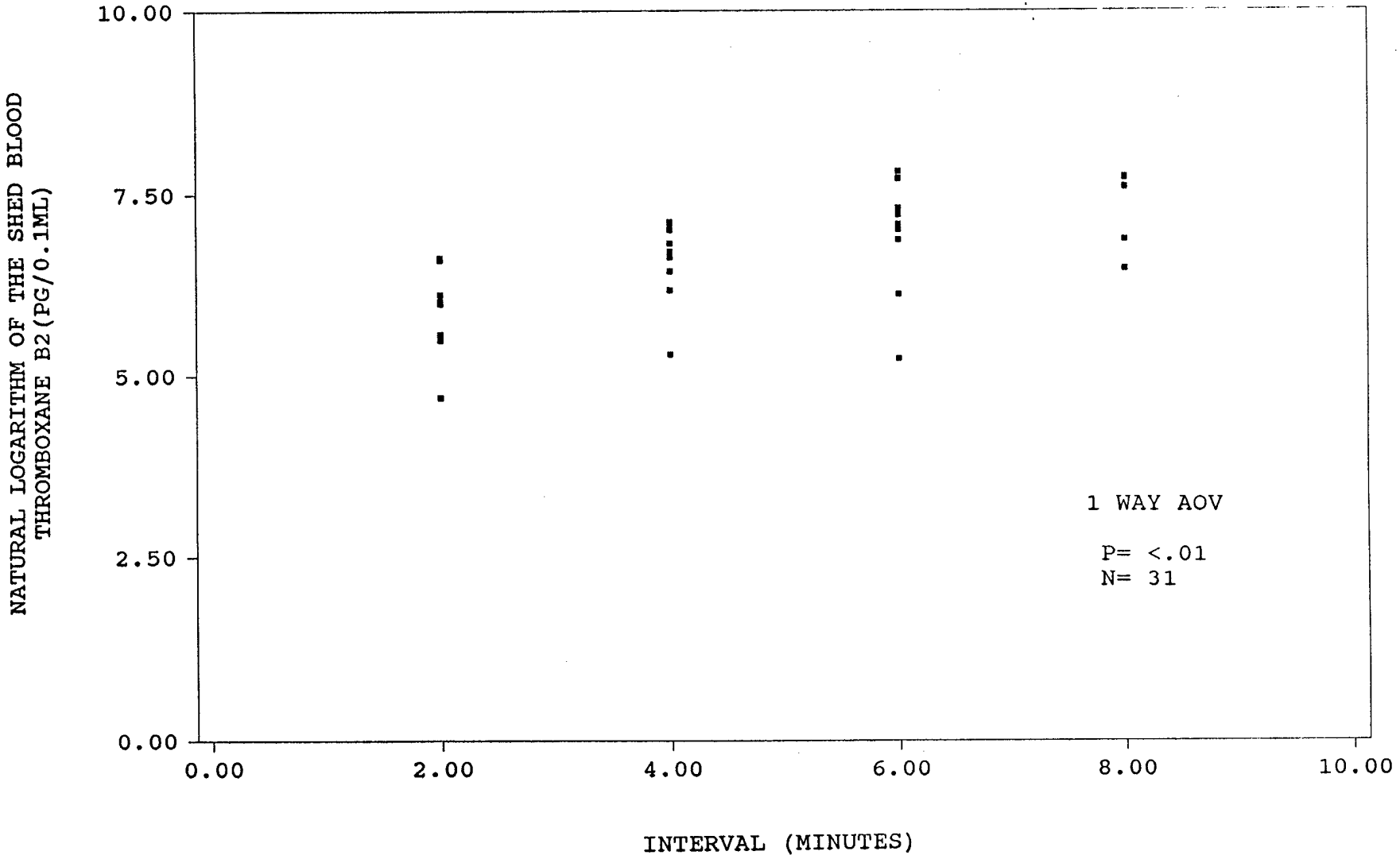
**THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN A 0.6 ML VOLUME OF SHED BLOOD; IN ALL THE SHED BLOOD FROM ONE BLEEDING SITE AND IN TWO MINUTE COLLECTION INTERVALS AT ONE BLEEDING TIME SITE AND THE LEVEL IN THE FIRST 2 MINUTE INTERVAL AND THE LAST 2 MINUTE INTERVAL REPORTED TOGETHER WITH THE BLEEDING TIME MEASURED AT THE LOCAL SKIN TEMPERATURE OF +32C**

| | Collection to 0.6 ml volume of shed blood pg/0.1 ml | Collection of all the shed blood from 1 site pg/0.1 ml | 2 Minute Collection intervals from 1 site Intervals First 2 min pg/0.1 ml | Last 2 min pg/0.1 ml | Mean BT (min) at 32C |
|---|---|---|---|---|---|
| Mean: | 6.333 | 7.010 | 5.857 | 7.185 | 2.047 |
| SD: | .520 | .704 | .601 | .643 | .333 |
| n: | 9 | 9 | 9 | 9 | 9 |

Paired T test between the first 2 minute and the last two minute sample <.001

## FIGURE 20

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED DURING THE BLEEDING TIME AT TWO MINUTE INTERVALS



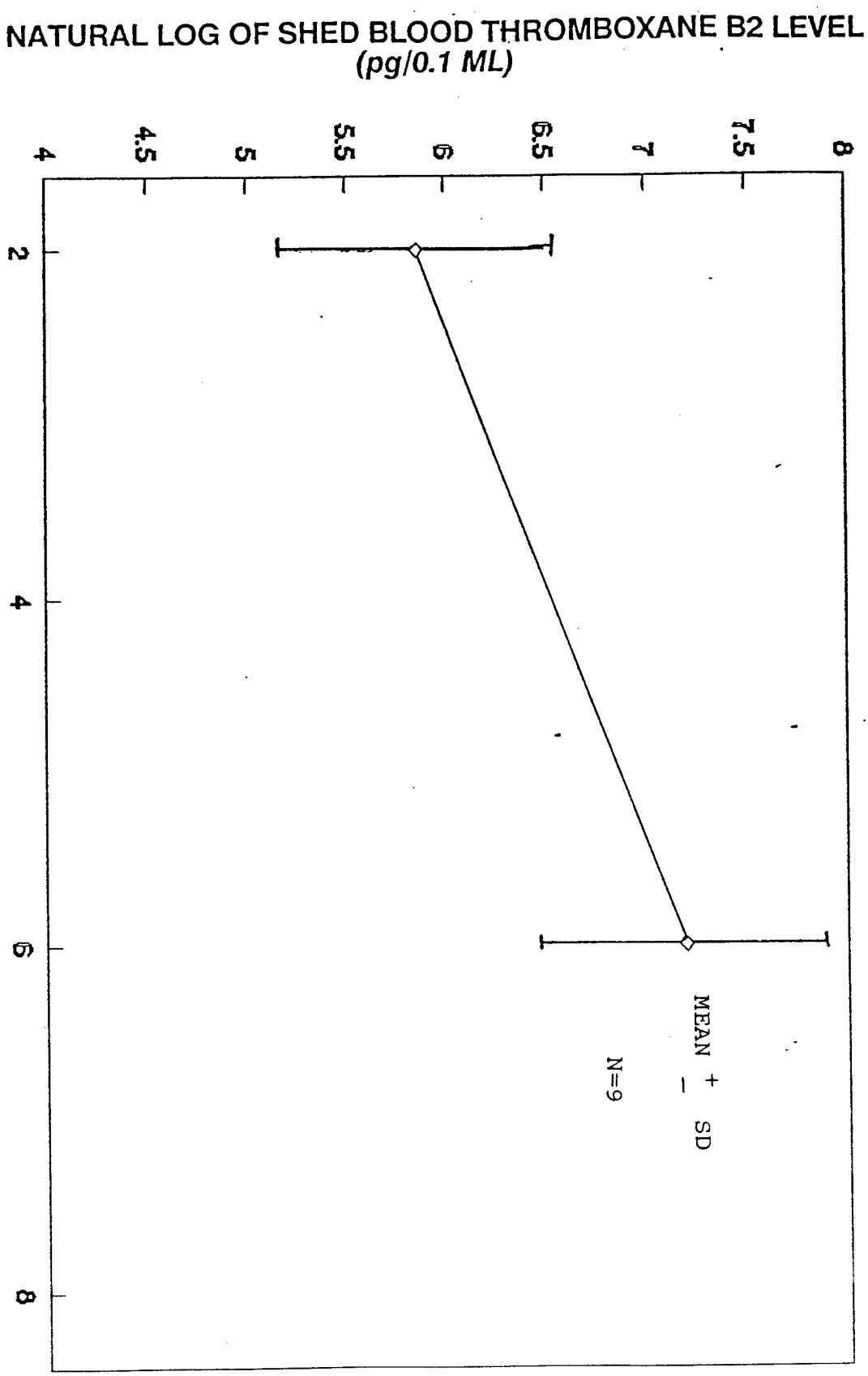



FIGURE 21

THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

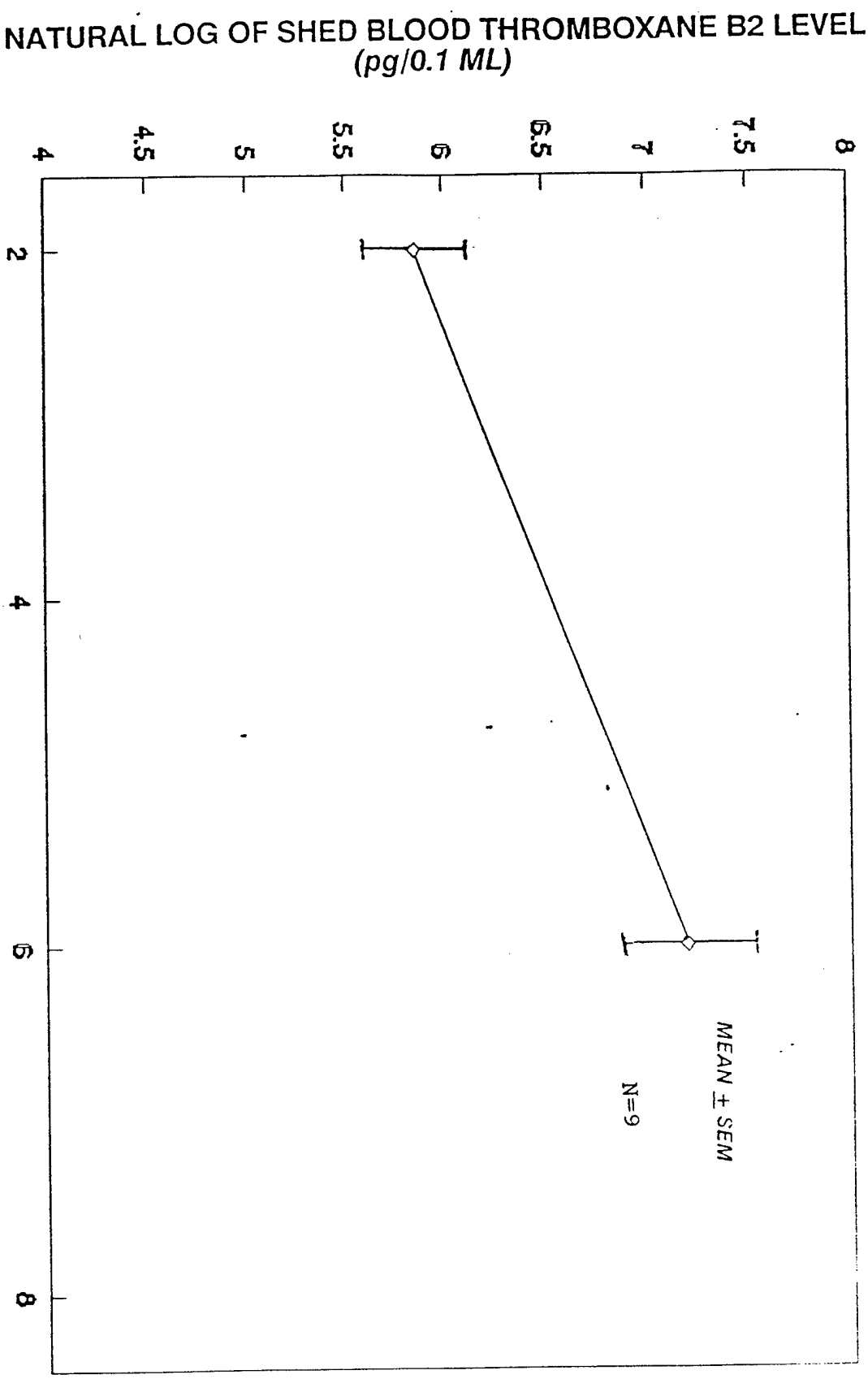



FIGURE 22

THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

TABLE 5A

SHED BLOOD TXB2 MEASUREMENTS IN BLOOD COLLECTED FROM A BLEEDING TIME SITE USING THREE METHODS OF COLLECTION AT THE LOCAL SKIN TEMPERATURES OF +32C, +28C, AND +22C

| | Collection of 0.6 ml shed blood (pg/0.1 ml) | Collected of all shed blood from BT site (pg/0.1 ml) | Shed blood collected for first 2 min from 1 site (pg/0.1 ml) | | Shed blood collected for last 2 min from 1 site (pg/0.1 ml) | BT in Min |
|---|---|---|---|---|---|---|
| **32C** | | | | | | |
| Mean: | 801 | 919 | 416 | | 1602 | 8.1 |
| SD: | 436 | 527 | 326 | | 1165 | 2.4 |
| n: | 6 | 6 | 6 | | 6 | 6 |
| | | | | =.05* | | |
| **28C** | | | | | | |
| Mean: | 572 | 439 | 339 | | 985 | 11.2 |
| SD: | 472 | 217 | 177 | | 595 | 3.1 |
| n: | 6 | 6 | 6 | | 6 | 6 |
| | | | | <.05* | | |
| **22C** | | | | | | |
| Mean: | 72 | 104 | 228 | | 129 | 23 |
| SD: | 35 | 92 | 194 | | 51 | 6 |
| n: | 5 | 5 | 5 | | 5 | 5 |
| | | | | NS | | |

* Paired T-test between first 2 minute collection and the last 2 minute collection.

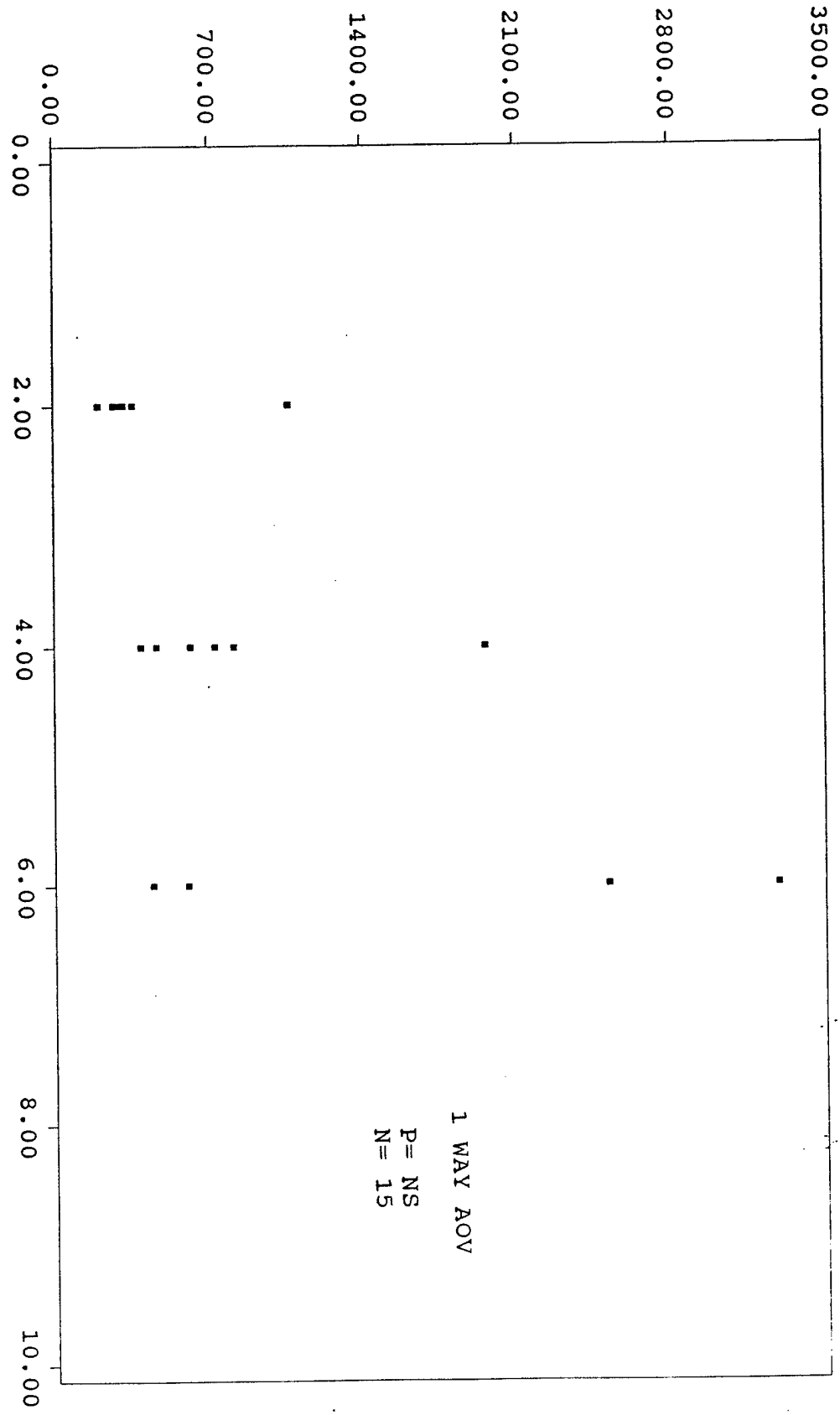



FIGURE 23

THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +32C

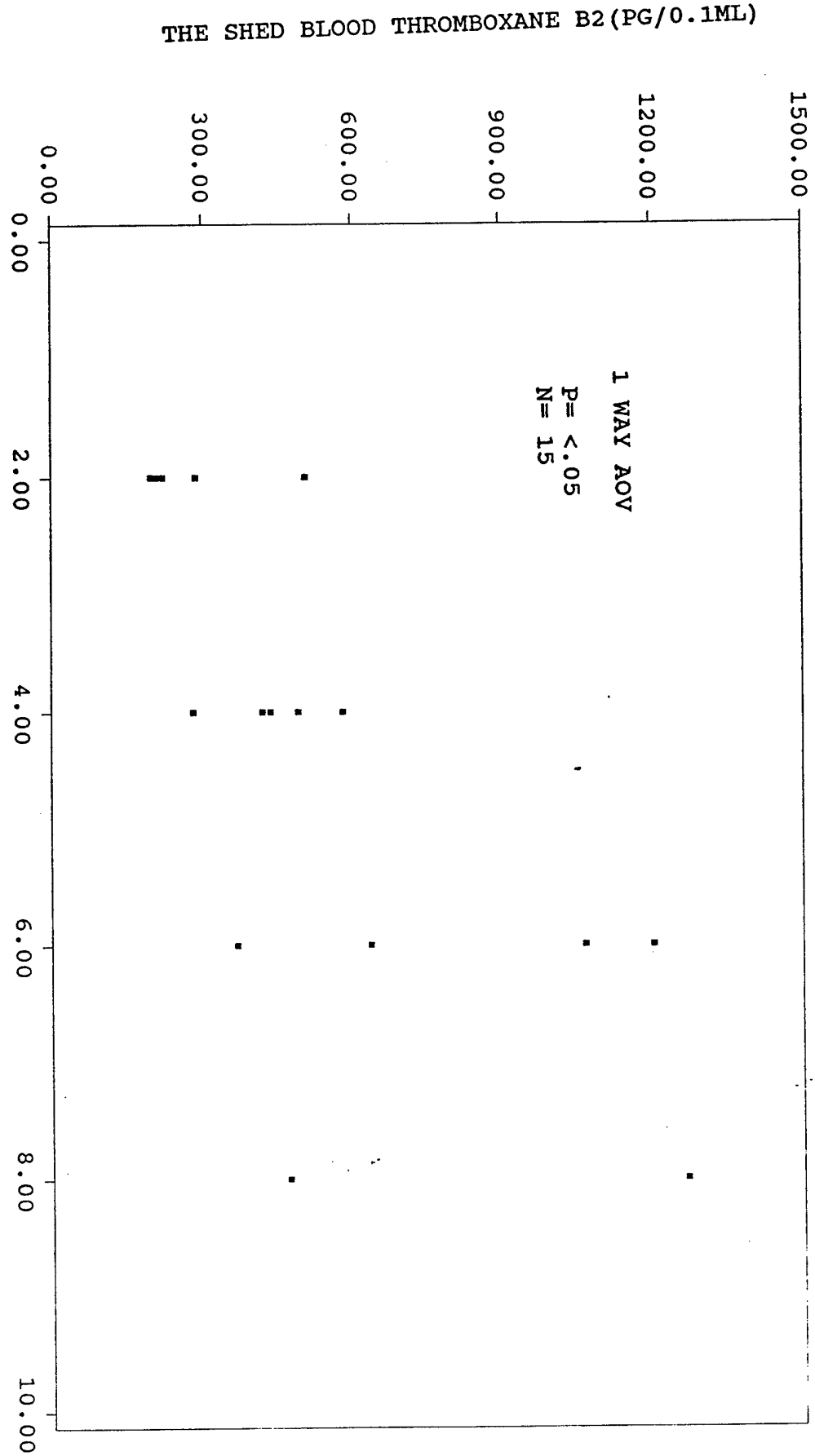
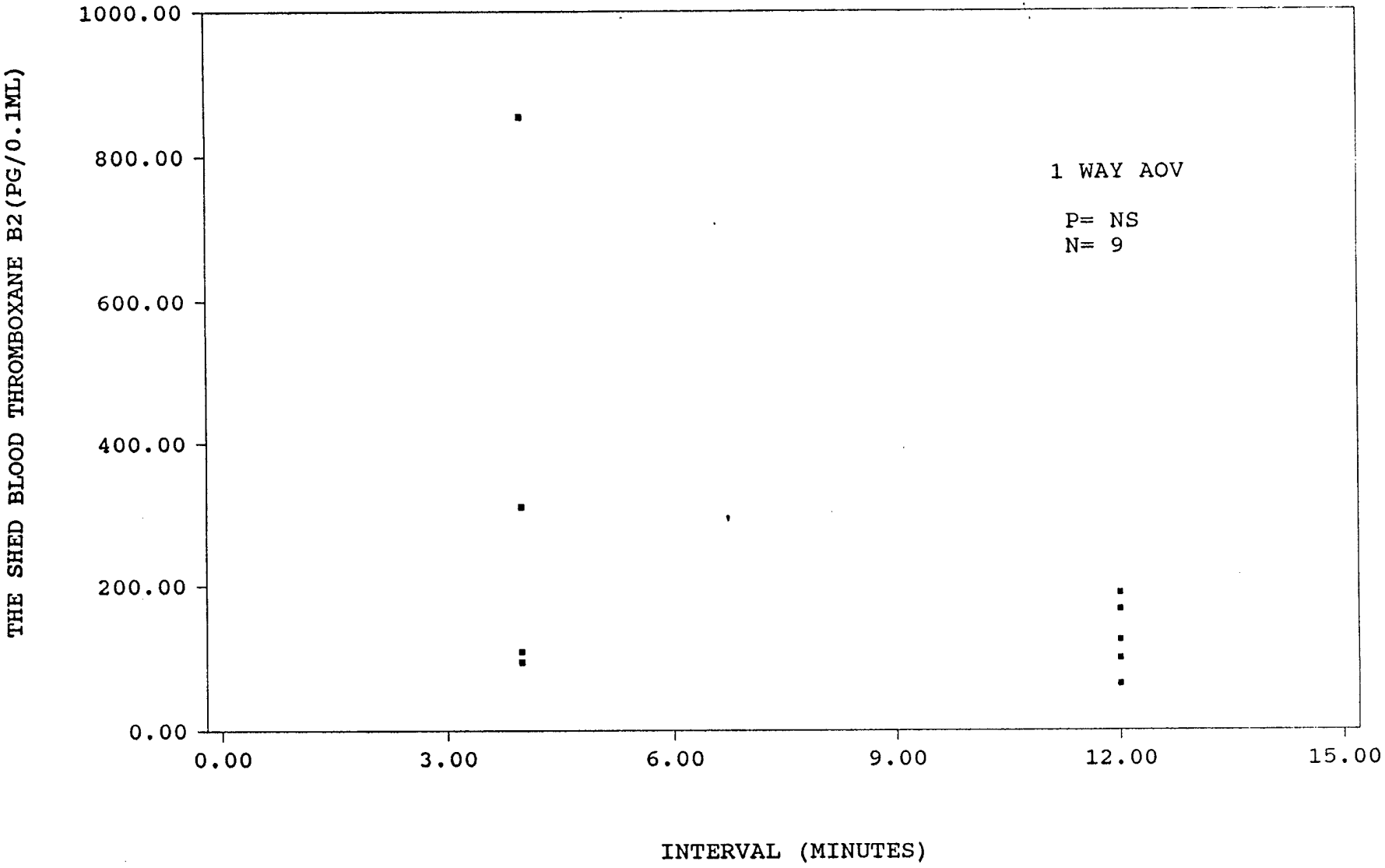



FIGURE 24

THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +28C

## FIGURE 25

**THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +22C**



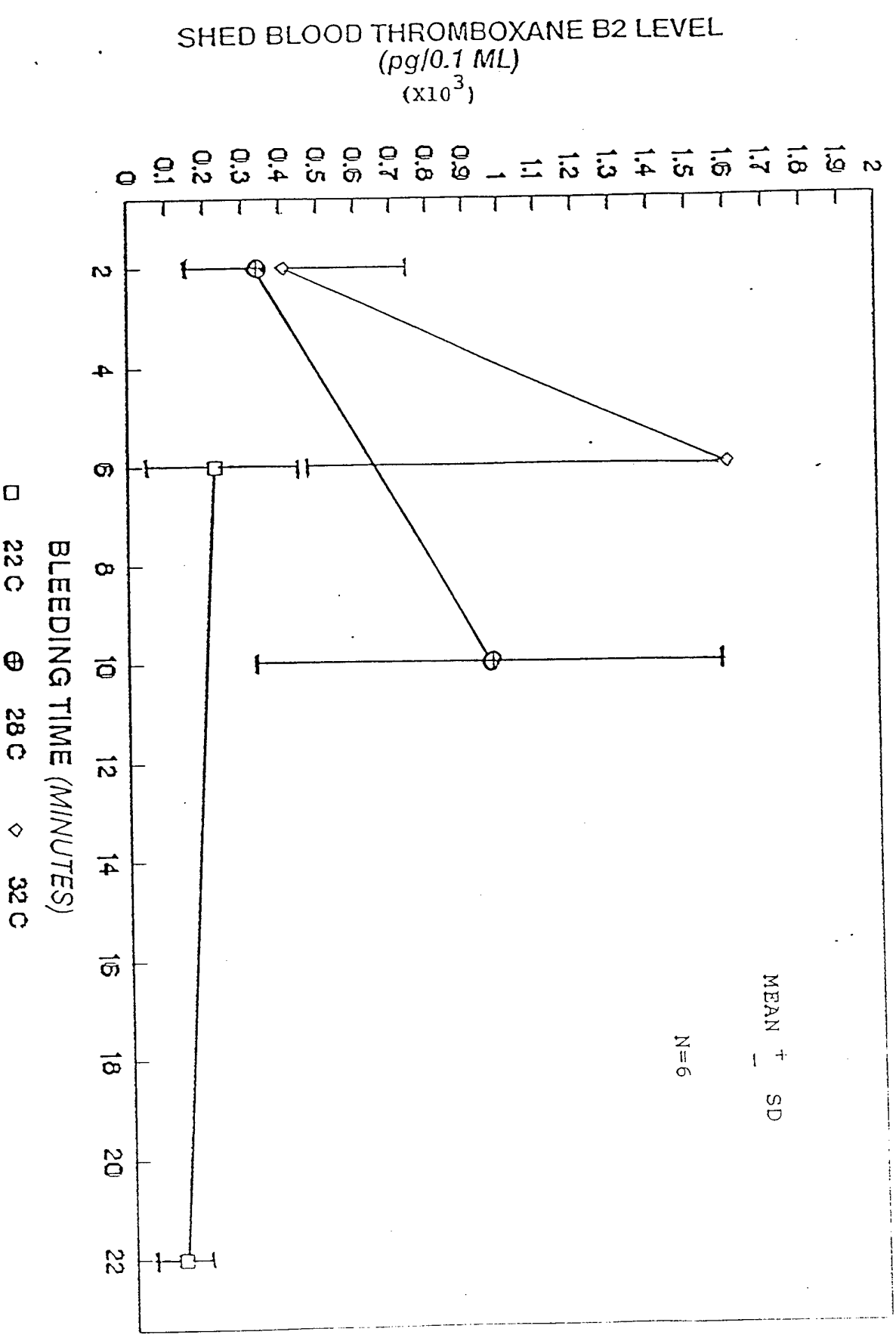



**FIGURE 26**

**THE THROMBOXANE $B_2$ LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME**

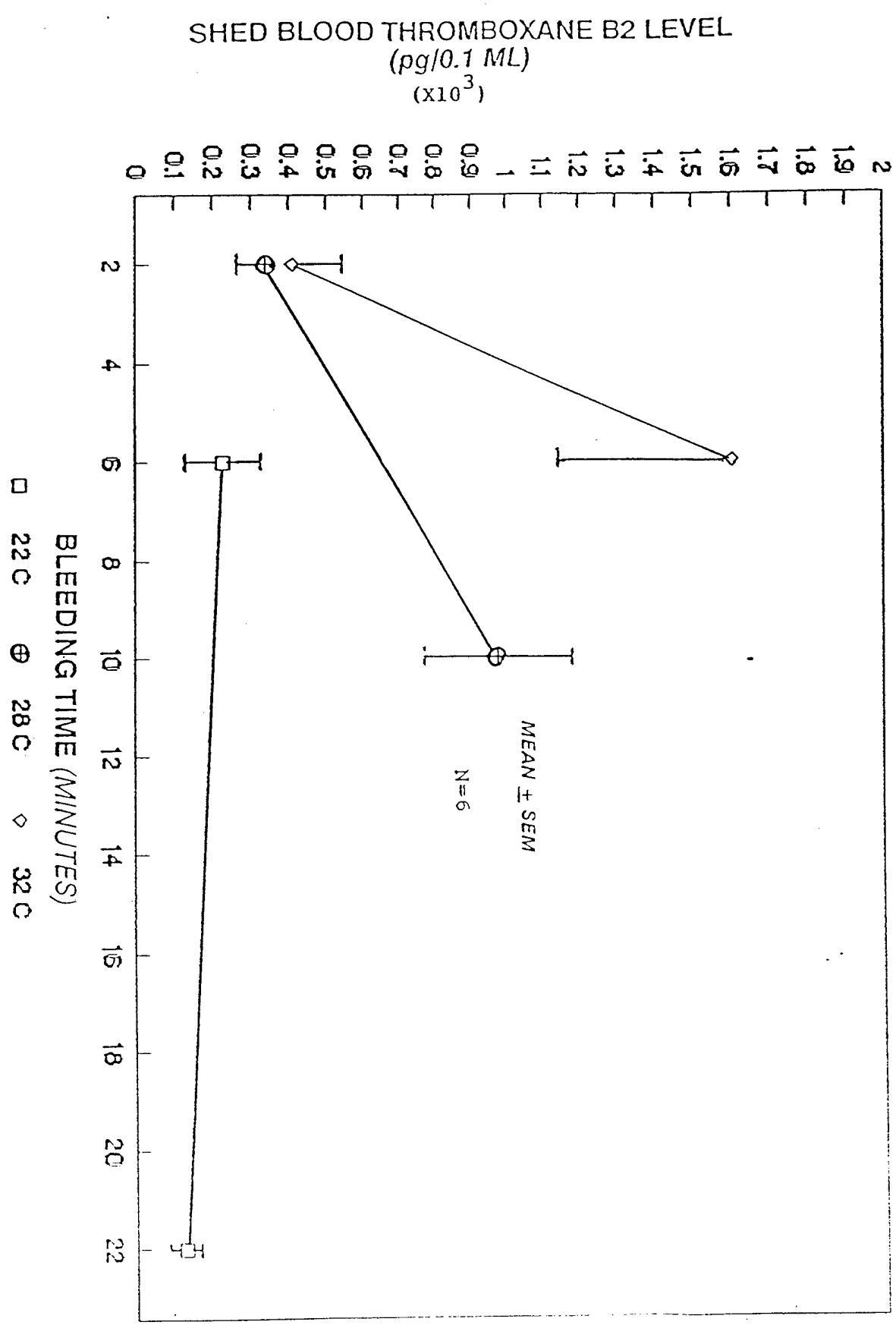



FIGURE 27

THE THROMBOXANE $B_2$ LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

TABLE 5B

**THE NATURAL LOGARITHM OF THE SHED BLOOD TXB2 MEASUREMENTS IN BLOOD COLLECTED FROM A BLEEDING TIME SITE USING THREE METHODS OF COLLECTION AT THE LOCAL SKIN TEMPERATURES OF +32C, +28C, AND +22C**

**N=6**

| | Collection of 0.6 ml shed blood (pg/0.1 ml) | Collected of all shed blood from BT site (pg/0.1 ml) | Shed blood collected for first 2 min from 1 site (pg/0.1 ml) | | Shed blood collected for last 2 min from 1 site (pg/0.1 ml) | BT in Min |
|---|---|---|---|---|---|---|
| **32C** | | | | | | |
| Mean: | 6.543 | 6.699 | 5.863 | | 7.115 | 2.047 |
| SD: | .610 | .537 | .575 | | .830 | .333 |
| n: | 6 | 6 | 6 | | 6 | 6 |
| Paired T*: | | | | <.05* | | |
| **28C** | | | | | | |
| Mean: | 6.114 | 5.66 | 5.722 | | 6.480 | 2.374 |
| SD: | .72 | .25 | .487 | | .713 | .302 |
| n: | 6 | 6 | 6 | | 6 | 6 |
| Paired T*: | | | | <.05* | | |
| **22C** | | | | | | |
| Mean: | 4.18 | 4.5 | 5.2 | | 4.78 | 3.13 |
| SD: | .47 | .7 | .99 | | .4 | .3 |
| n: | 5 | 5 | 5 | | 5 | 5 |
| | | | | NS* | | |

* Paired T-test between first 2 minute collection and the last 2 minute collection.

FIGURE 28

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED AT TWO MINUTE INTERVAL DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +32C

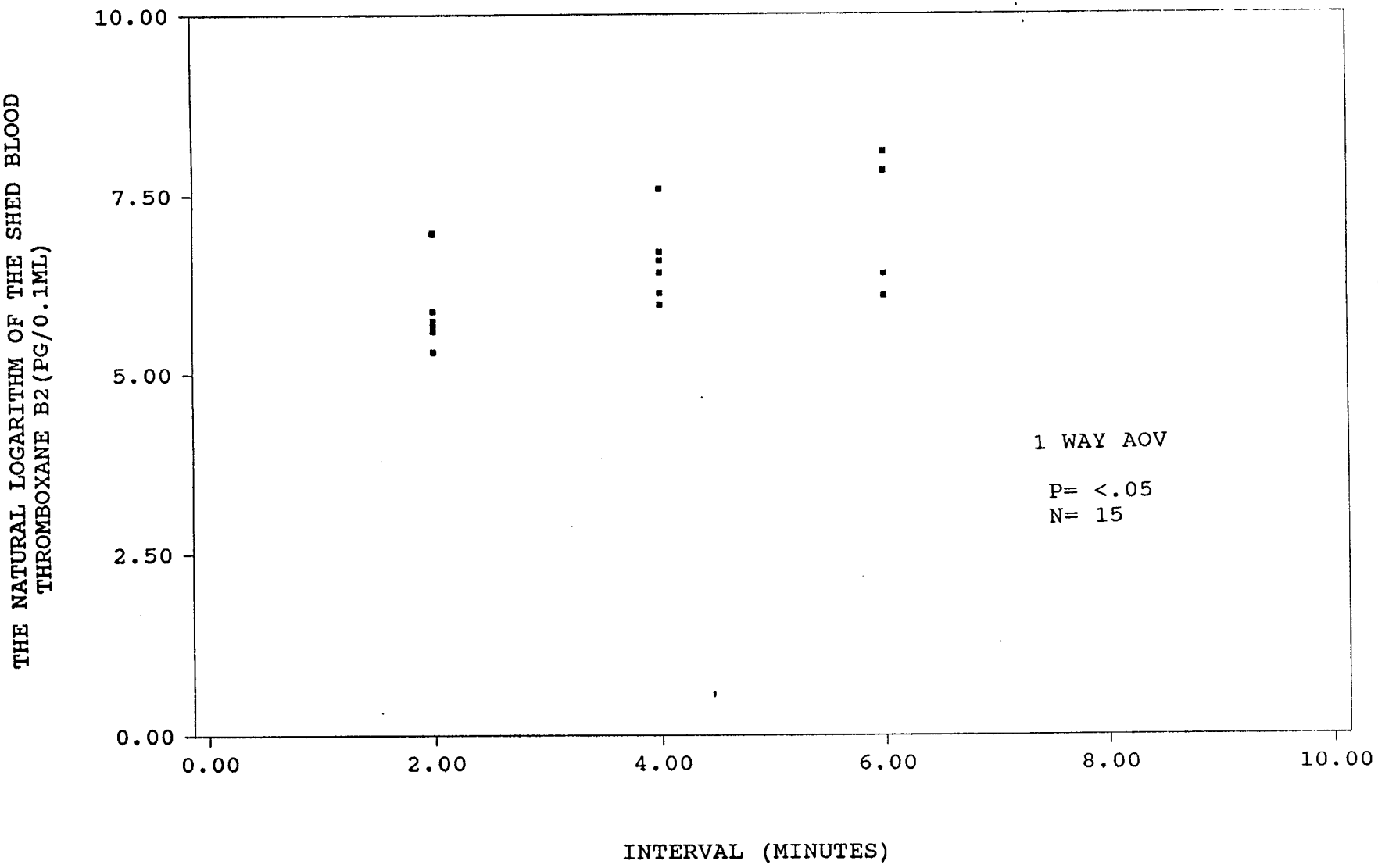


FIGURE 29

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE THE SKIN TEMPERATURE WAS +28C

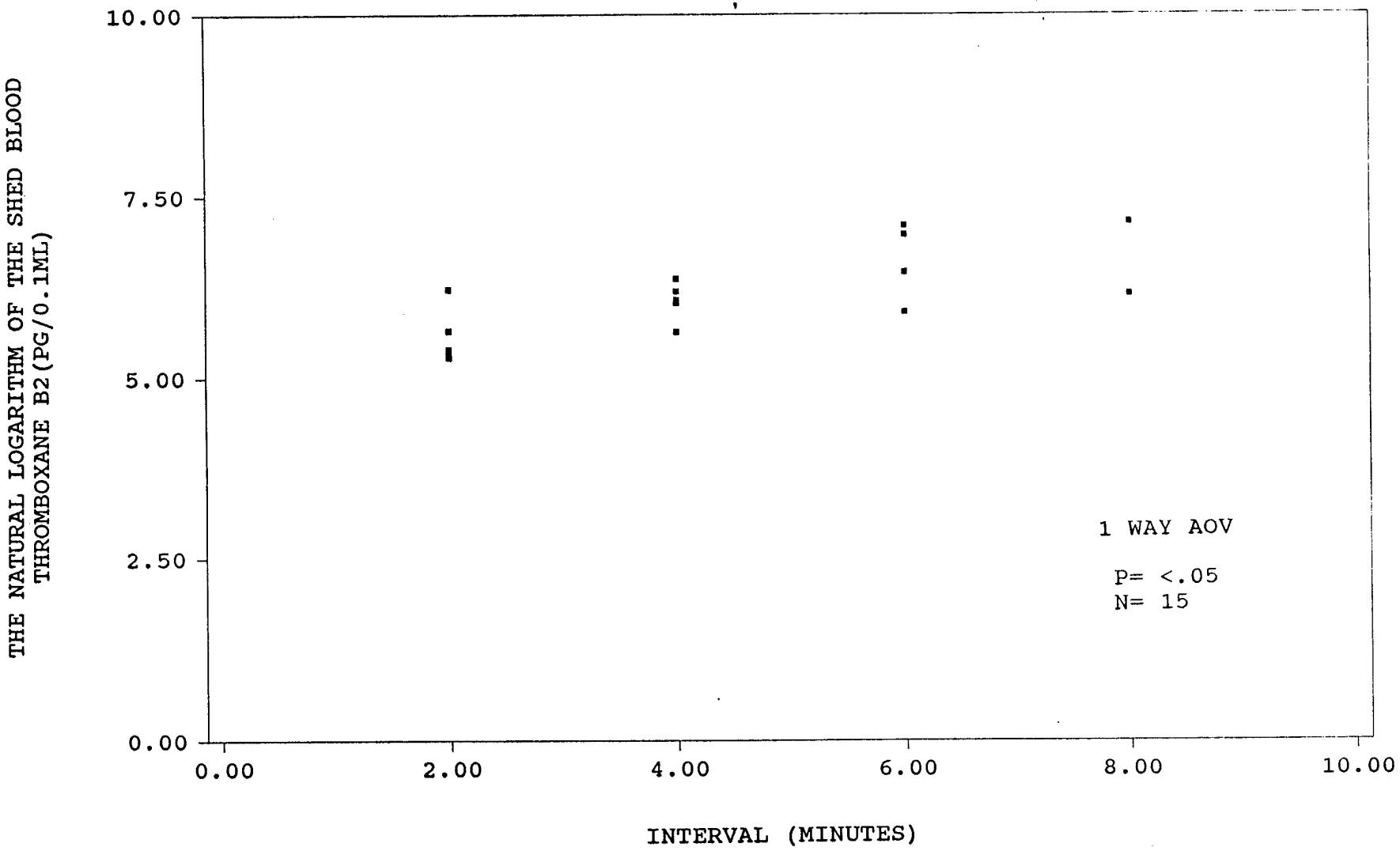
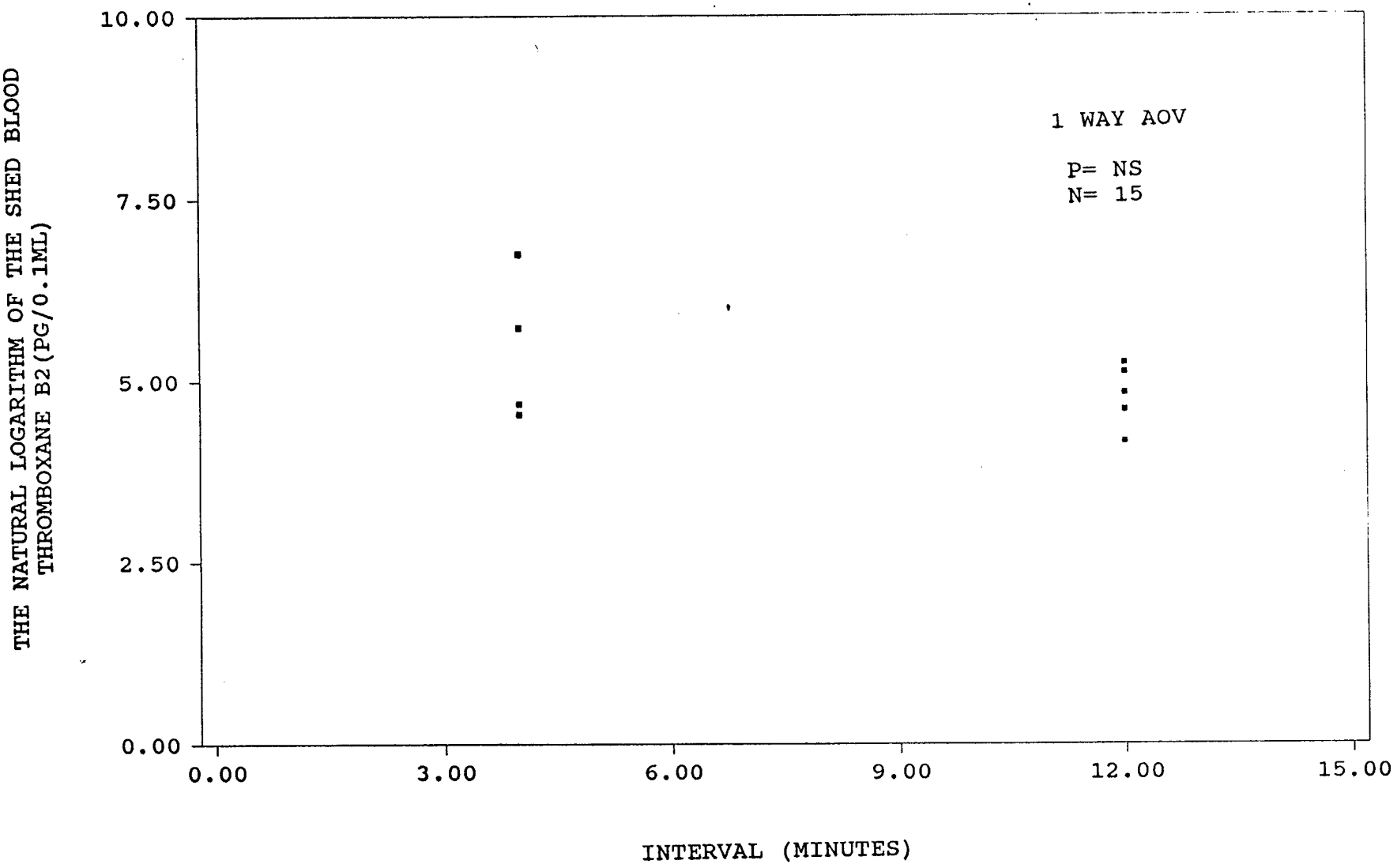


FIGURE 30

THE NATURAL LOGARITHM OF THE SHED BLOOD THROMBOXANE $B_2$ LEVEL MEASURED AT TWO MINUTE INTERVALS DURING THE BLEEDING TIME WHEN THE SKIN TEMPERATURE WAS +22C



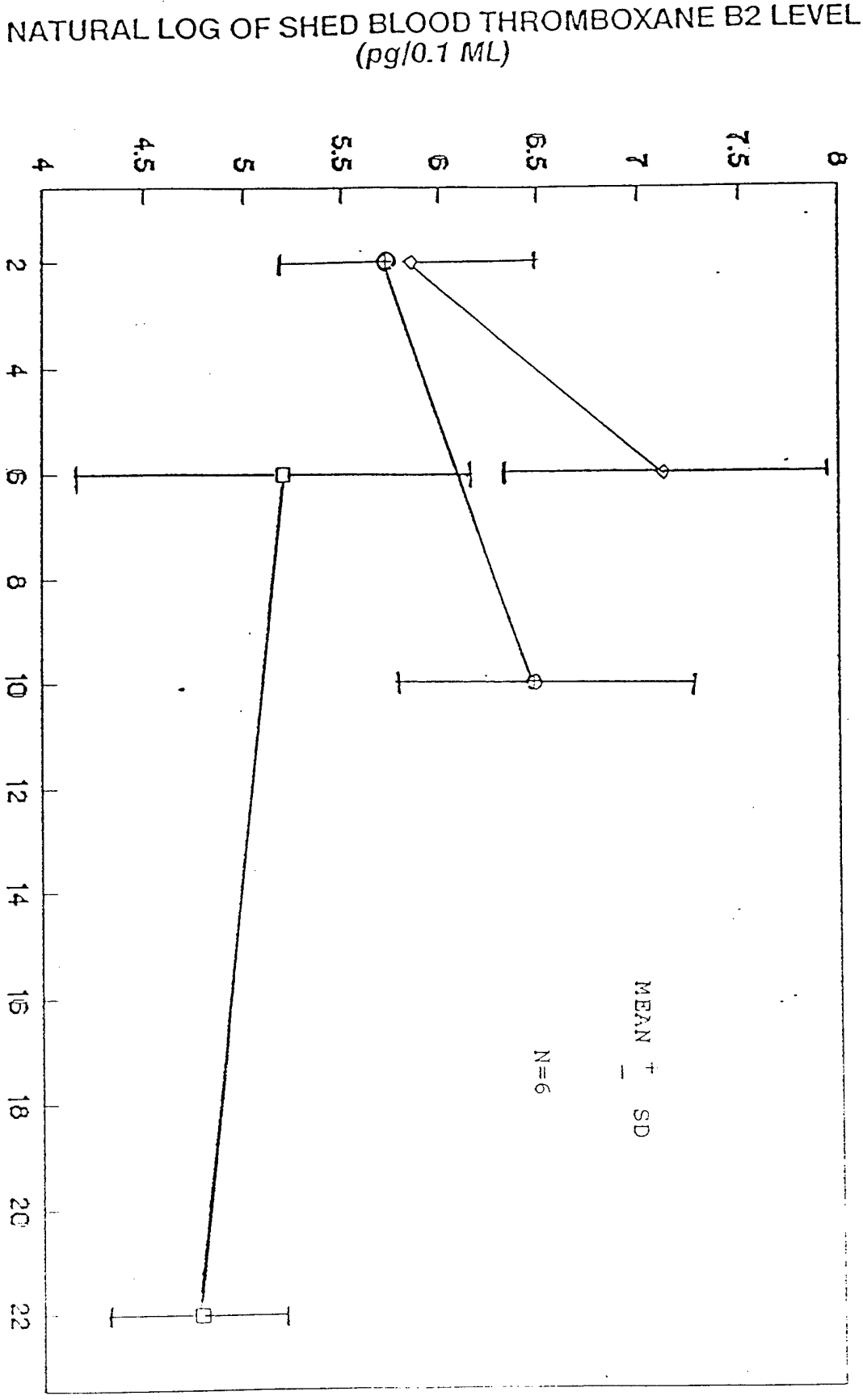



**FIGURE 31**

**THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME**

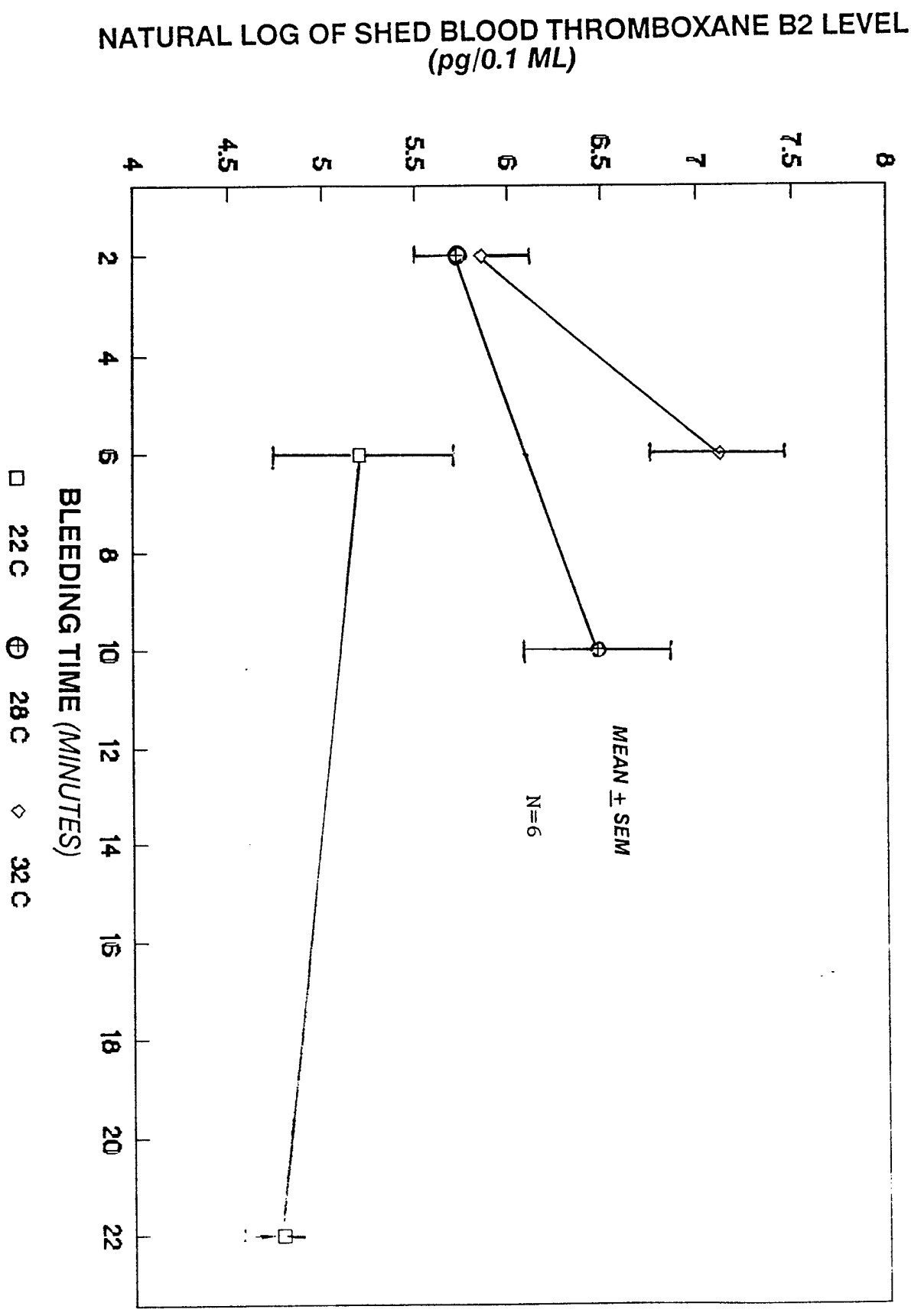



FIGURE 32

THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN THE SHED BLOOD COLLECTED IN INTERVALS DURING THE MEASUREMENT OF THE BLEEDING TIME

TABLE 6

**IN VITRO CLOTTING TIMES AND THROMBOXANE $B_2$ LEVELS THE SERUM OBTAINED FROM 7 ML OF BLOOD CLOTTED WITH AGITATION IN 7.5 ML TUBES AT FOUR TEMPERATURES IN VITRO**

| n=3 Waterbath Temp | 37C | 32C | 28C | 22C |
|---|---|---|---|---|
| Clotting Time (min) | | | | |
| 1 | 3.5 | 4.5 | 7.5 | 15 |
| 2 | 3.0 | 3.5 | 6.5 | 15 |
| 3 | 5.0 | 5.5 | 6.5 | 13.5 |
| Mean | 3.8 | 4.5 | 6.8 | 14.5 |
| SD | 1.0 | 1.0 | 0.6 | 1.0 |
| Serum TXB2 pg/.01 ml | | | | |
| 1 | 20200 | 1880 | 106 | 47 |
| 2 | 15000 | 1780 | 608 | 65 |
| 3 | 12200 | 3498 | 475 | 127 |
| Mean | 15800 | 2386 | 396 | 60 |
| SD | 4060 | 964 | 260 | 62 |

TABLE 7

**IN VITRO CLOTTING TIMES AND THROMBOXANE $B_2$ LEVELS IN THE SERUM OBTAINED FROM 3 ML OF BLOOD CLOTTED WITH AGITATION IN 3.5 ML TUBES AT FOUR TEMPERATURES IN VITRO**

| n=3 | Waterbath Temp | 37C | 32C | 28C | 22C | |
|---|---|---|---|---|---|---|
| | Clotting Time (min) | | | | | |
| | 1 | | | 1 | 3.0 | 3.5 |
| | 2 | 3.5 | 4.5 | 5.5 | 7.5 | |
| | 3 | 3.5 | 4.5 | 5.5 | 8.0 | |
| | Mean | 3.3 | 4.2 | 5.5 | 8.0 | |
| | SD | .3 | .6 | 0 | .5 | |
| | Serum TXB2 pg/.01 ml | | | | | |
| | 1 | 46789 | 13795 | 2565 | 509 | |
| | 2 | 2385 | 640 | 1625 | 2301 | |
| | 3 | 1863 | 1593 | 824 | 1170 | |
| | Mean | 17000 | 5339 | 1338 | 1327 | |
| | SD | 26000 | 7300 | 1067 | 906 | |

TABLE 8

N=2

**IN VITRO CLOTTING TIMES AND THROMBOXANE $B_2$ LEVELS IN THE SERUM OBTAINED FROM 3.0 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO**

| Waterbath Temp | 37C | | 32C | | 28C | | 22C | |
|---|---|---|---|---|---|---|---|---|
| Clotting Time (min) | | | | | | | | |
| 1 | 4.0 | | 4.5 | | 5.0 | | 9.5 | |
| 2 | 4.5 | | 5.5 | | 6.0 | | 8.0 | |
| 3 | -- | | -- | | -- | | -- | |
| Mean | 4.25 | | 5.0 | | 5.5 | | 8.8 | |
| SD | .35 | | .7 | | .7 | | 1.1 | |
| Serum TxB pg/.01 ml | A+ | S++ | A | S | A | S | A | S |
| 1 | 2310 | 1015 | 2310 | 335 | 1503 | 629 | 657 | 297 |
| 2 | 1425 | 318 | 342 | 321 | 402 | 862 | 73 | 65 |
| 3 | -- | -- | -- | -- | -- | -- | -- | -- |
| Mean | 1868 | 667 | 1326 | 520 | 953 | 746 | 365 | 181 |
| SD | 620 | 493 | 1392 | 971 | 798 | 165 | 413 | 164 |

+Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample

TABLE 9

**IN VITRO CLOTTING TIMES AND THROMBOXANE $B_2$ LEVELS IN THE SERUM OBTAINED FROM 7 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 7.5 ML TUBE AT FOUR TEMPERATURES IN VITRO**

n=2

| Waterbath Temp | 37C | | 32C | | 28C | | 22C | |
|---|---|---|---|---|---|---|---|---|
| Clotting Time (min) | | | | | | | | |
| 1 | 6.0 | | 6.5 | | 11.5 | | 41.50+++ | |
| 2 | 6.0 | | 6.5 | | 12.0 | | 30.0 | |
| 3 | -- | | -- | | -- | | -- | |
| Mean | 6.0 | | 6.5 | | 11.8 | | 36 | |
| SD | 0 | | 0 | | .4 | | 8 | |
| Serum TXB pg/.01 ml | A+ | S++ | A | S | A | S | A | S |
| 1 | 3435 | 809 | 931 | 520 | 135 | 91 | 57 | 42 |
| 2 | 9615 | 931 | 6385 | 971 | 422 | 218 | 51 | 30 |
| 3 | -- | -- | -- | -- | -- | -- | -- | -- |
| Mean | 6525 | 890 | 3658 | 746 | 279 | 155 | 54 | 36 |
| SD | 4370 | 86 | 3857 | 319 | 203 | 90 | 4 | 8 |

+Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample
+++ No clot formed

TABLE 10

**IN VITRO CLOTTING TIMES AND THROMBOXANE $B_2$ LEVELS IN THE SERUM OBTAINED FROM 1 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO**

n=3

| Waterbath Temp | 37C | | 32C | | 28C | | 22C | |
|---|---|---|---|---|---|---|---|---|
| Clotting Time (min) | | | | | | | | |
| 1 | 2.5 | | 3.0 | | 5.0 | | 6.5 | |
| 2 | 3.0 | | 3.5 | | 4.0 | | 6.5 | |
| 3 | 2.5 | | 3.0 | | 3.5 | | 6.5 | |
| Mean | 1.7 | | 3.2 | | 4.2 | | 6.5 | |
| SD | 1.0 | | 0.7 | | 0.8 | | 0 | |
| Serum TXB pg/.01 ml | A+ | S++ | A | S | A | S | A | S |
| 1 | 7373 | 3721 | 4000 | 1174 | 559 | 803 | 495 | -- |
| 2 | 21320 | 4110 | 196000 | 2650 | 16170 | 1745 | 1853 | 1169 |
| 3 | 4694 | 598 | 1354 | 570 | 1234 | 512 | 2468 | 242 |
| Mean | 11129 | 2810 | 8318 | 1464 | 5988 | 1020 | 1605 | |
| SD | 8926 | 1925 | 9860 | 1070 | 8825 | 645 | 1010 | |

+Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample

TABLE 11

**IN VITRO CLOTTING TIMES AND THROMBOXANE B2 LEVELS IN THE SERUM OBTAINED FROM 1 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO**

n=3

| Waterbath Temp | 37C | | 32C | | 28C | | 22C | |
|---|---|---|---|---|---|---|---|---|
| Clotting Time (min) | | | | | | | | |
| 1 | 2.5 | | 3.0 | | 3.5 | | 6.0 | |
| 2 | 3.0 | | 4.0 | | 5.0 | | 6.5 | |
| 3 | 2.5 | | 3.5 | | 4.5 | | 5.0 | |
| Mean | 2.7 | | 3.5 | | 4.3 | | 5.8 | |
| SD | .3 | | .5 | | .8 | | .8 | |
| Serum TXB pg/.01 ml | A+ | S++ | A | S | A | S | A | S |
| 1 | 2958 | 559 | 1232 | 259 | 806 | 246 | 447 | 256 |
| 2 | 5958 | 828 | 3810 | 311 | 1171 | 250 | 354 | 125 |
| 3 | 5520 | 603 | 1336 | 228 | 820 | 610 | 611 | 250 |
| Mean | 4812 | 663 | 2126 | 266 | 932 | 369 | 471 | 177 |
| SD | 1620 | 144 | 1459 | 42 | 207 | 209 | 130 | 132 |

\+ Agitated for the measurement of clotting time
++Stationary for the period of time to clot the agitated sample

TABLE 12

**IN VITRO CLOTTING TIME AND THROMBOXANE $B_2$ LEVELS IN THE SERUM OBTAINED FROM 1.0 ML OF BLOOD CLOTTED WITH AND WITHOUT AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO**

n=6

| Waterbath Temp | 37C | 32C | 28C | 22C |
|---|---|---|---|---|
| Clotting Time (min) | 2.5 | 3.0 | 5.0 | 6.5 |
| | 3.0 | 3.5 | 4.0 | 6.5 |
| | 2.5 | 3.0 | 3.5 | 6.5 |
| | 2.5 | 3.0 | 3.5 | 6.0 |
| | 3.0 | 4.0 | 5.0 | 6.5 |
| | 2.5 | 3.5 | 4.5 | 5.0 |
| Mean: | 2.7 | 3.33 | 4.3 | 6.2 |
| SD: | .3 | .4 | .7 | .6 |

| Serum TXB pg/.01 ml | A+ | S++ | A | S | A | S | A | S |
|---|---|---|---|---|---|---|---|---|
| | 7373 | 3721 | 4000 | 1174 | 559 | 803 | 495 | -- |
| | 21302 | 4110 | 19600 | 2650 | 16170 | 1745 | 1853 | 1169 |
| | 4694 | 598 | 1354 | 570 | 1234 | 512 | 2468 | 242 |
| | 2958 | 559 | 1232 | 259 | 806 | 246 | 447 | 256 |
| | 5958 | 828 | 3810 | 311 | 1171 | 250 | 354 | 125 |
| | 5520 | 603 | 1336 | 228 | 820 | 610 | 611 | 250 |
| Mean: | 7967 | 1737 | 5222 | 865 | 3460 | 694 | 1038 | 389 |
| SD: | 6693 | 1695 | 7158 | 943 | 6232 | 558 | 895 | 447 |

\+ Agitated for the measurement of clotting time.
++ Stationary for the period of time to clot the agitated sample.

TABLE 13

**IN VITRO CLOTTING TIMES AND THROMBOXANE $B_2$ LEVELS IN THE SERUM OBTAINED FROM 1 ML OF BLOOD CLOTTED WITH AGITATION IN A 3.5 ML TUBE AT FOUR TEMPERATURES IN VITRO**

| Waterbath Temp | 37C | 32C | 28C | 22C |
|---|---|---|---|---|
| Clotting Time (min) | | | | |
| Mean: | 2.7 | 3.33 | 4.3 | 6.2 |
| SD: | 0.3 | 0.4 | 0.7 | 0.6 |
| n: | 6 | 6 | 6 | 6 |
| Serum TXB pg/.01 ml | | | | |
| Mean: | 7967 | 5222 | 3460 | 1038 |
| SD: | 6693 | 7158 | 6232 | 895 |
| n: | 6 | 6 | 6 | 6 |

TABLE 14A

THE IN VITRO CLOTTING TIME AND THROMBOXANE B2 LEVELS IN THE SERUM OBTAINED FROM AGITATED OR NON-AGITATED WHOLE BLOOD CLOTTED AT FOUR TEMPERATURES

| Waterbath Temp | 37C | 32C | 28C | 22C | 1 Way AOV |
|---|---|---|---|---|---|
| Clotting Time (min) | | | | | |
| Mean | 3.6 | 4.3 | 6.1 | 12.1 | <.0001 |
| SD | 1.2 | 1.2 | 2.5 | 10.0 | |
| n | 16 | 16 | 16 | 16 | |
| Serum TXB2 pg/0.1 ml from agitated whole blood | | | | | |
| Mean | 10190 | 4030 | 1839 | 705 | <.0001 |
| SD | 11650 | 5286 | 3873 | 812 | |
| n | 16 | 16 | 16 | 16 | |
| Serum TXB2 pg/0.1 ml from non-agitated whole blood | | | | | |
| Mean | 1349 | 734 | 597 | 275 | NS |
| SD | 1371 | 744 | 481 | 350 | |
| n | 10 | 10 | 10 | 10 | |
| Paired T between agitated whole blood and non-agitated whole blood | <.01 | <.05 | NS | NS | |

FIGURE 33

THE RELATIONSHIP BETWEEN THE CLOTTING TIME IN THE AGITATED WHOLE BLOOD AND FOUR BLOOD TEMPERATURES

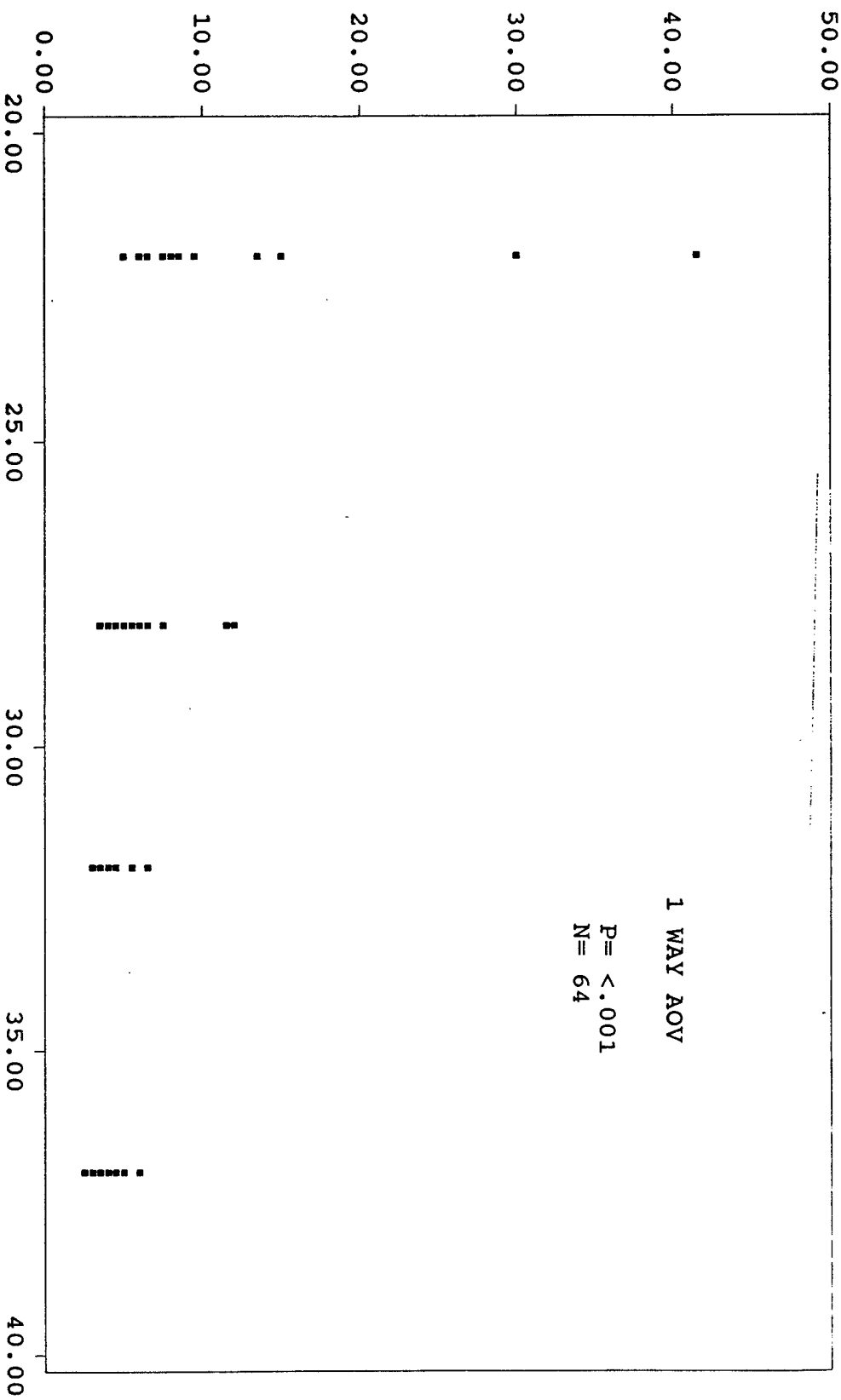


FIGURE 34

THE RELATIONSHIP OF THE SERUM THROMBOXANE $B_2$ LEVELS IN AGITATED CLOTTED BLOOD AND FOUR BLOOD TEMPERATURES

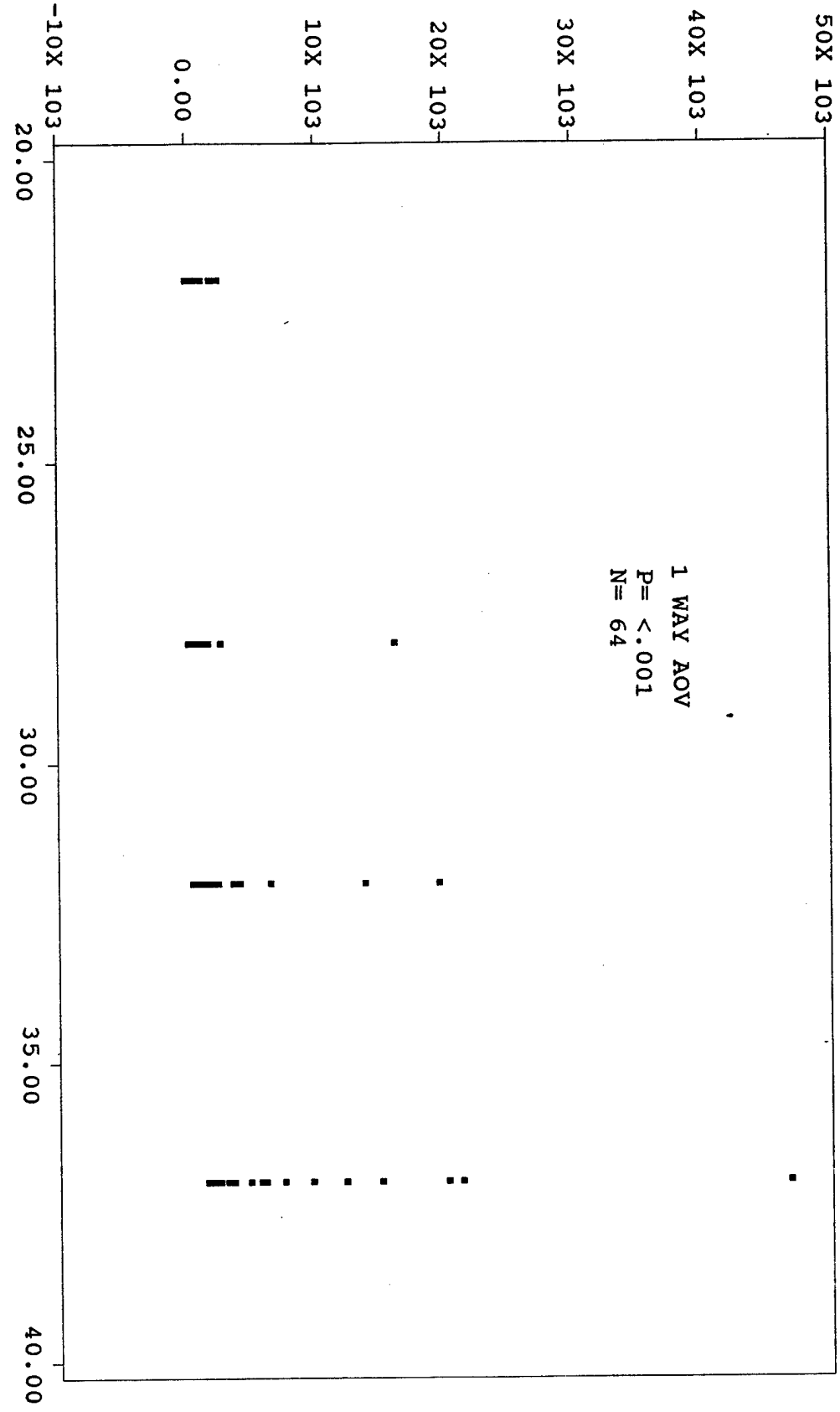


FIGURE 35

THE RELATIONSHIP BETWEEN THE SERUM THROMBOXANE $B_2$ LEVELS IN NON AGITATED CLOTTED BLOOD AND FOUR BLOOD TEMPERATURES

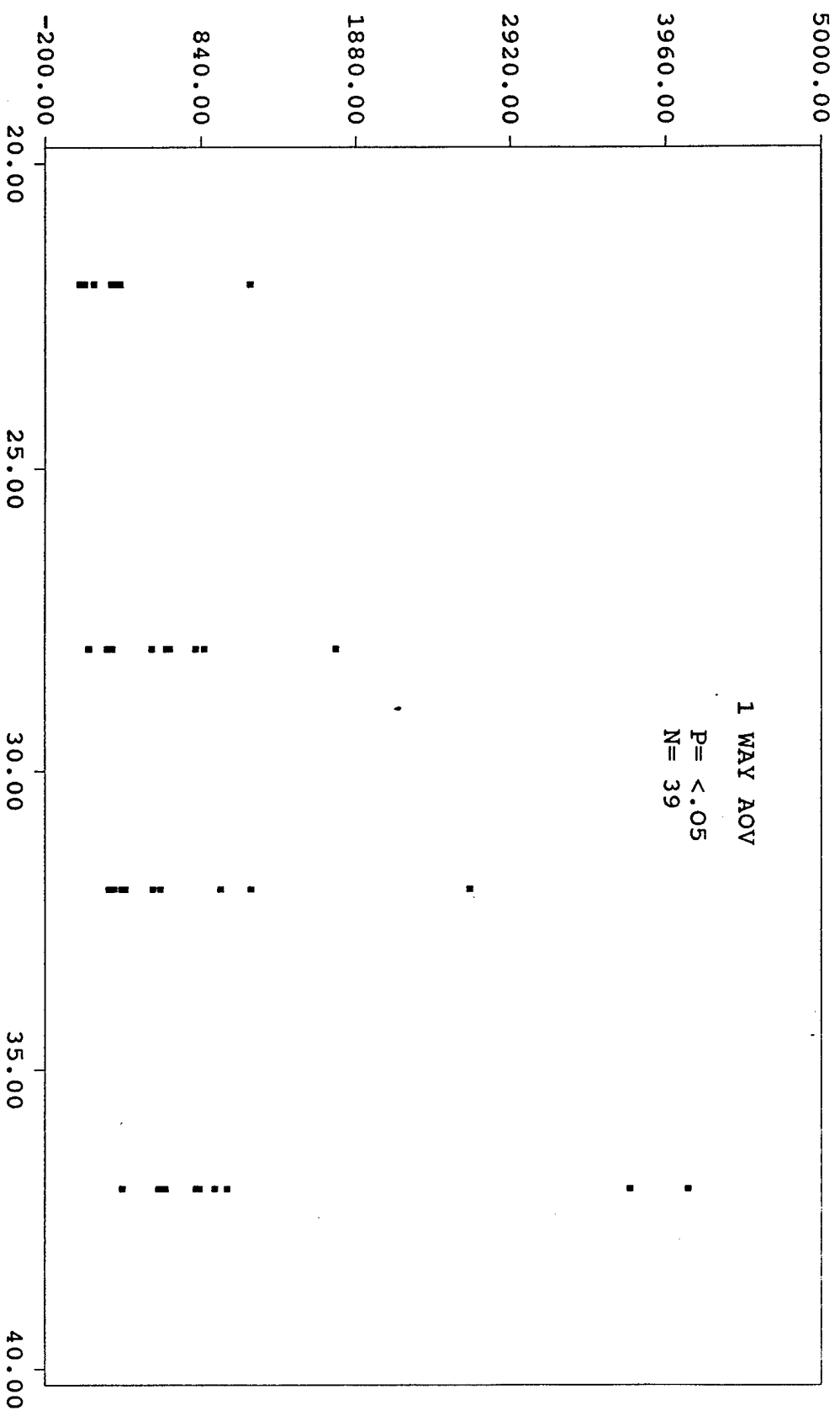


FIGURE 36

THE CLOTTING TIME IN MINUTES OF AGITATED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

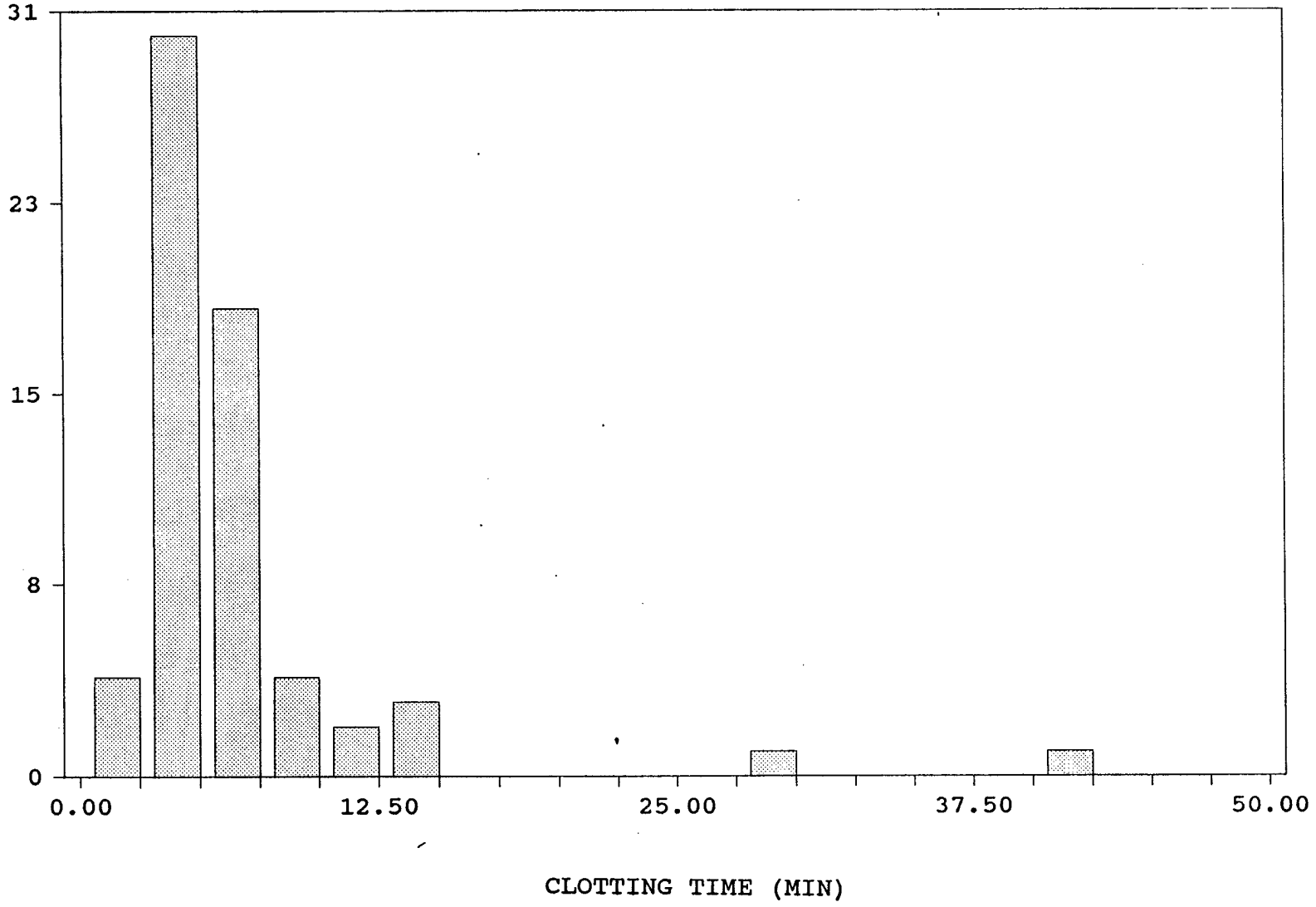
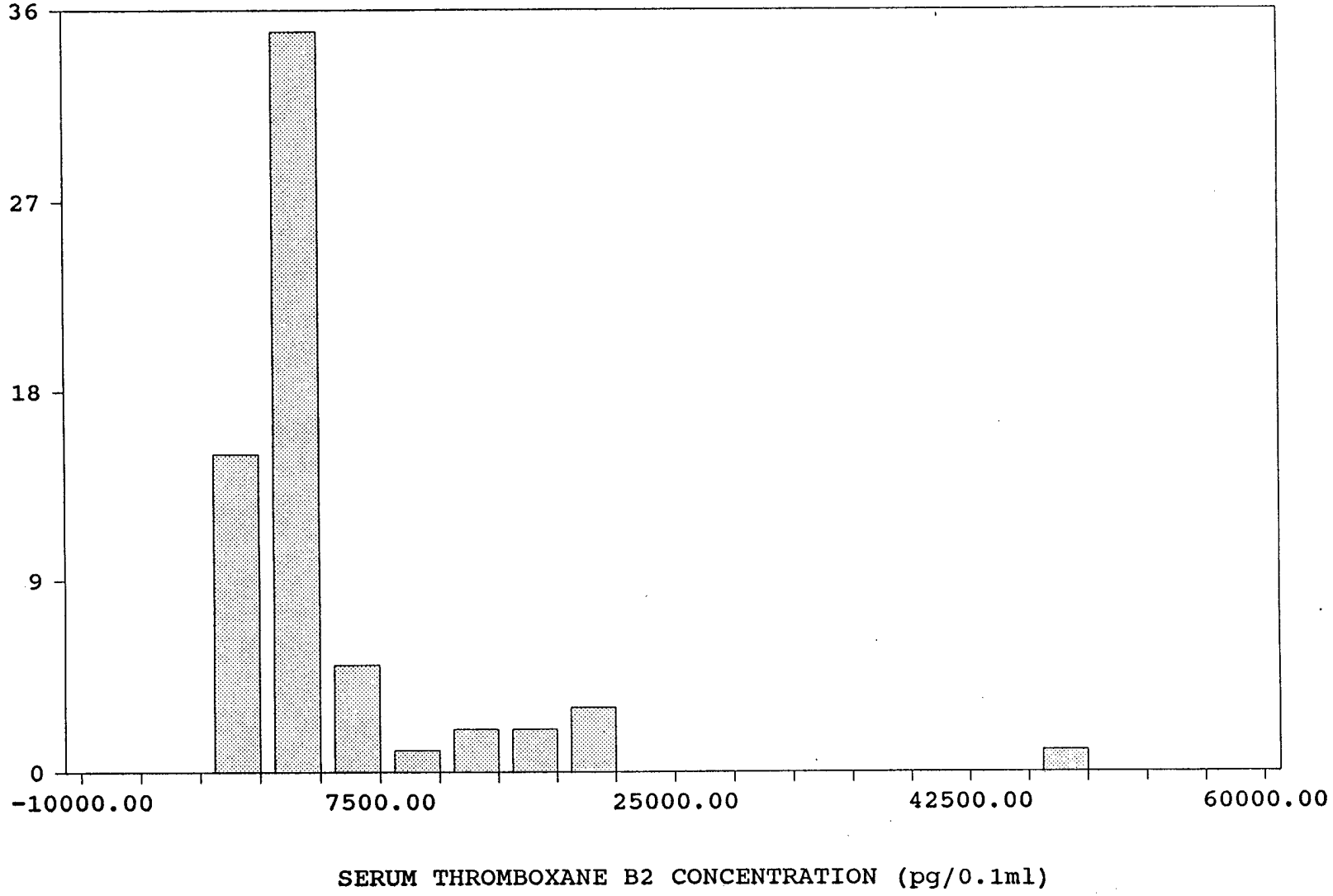


FIGURE 37

THE THROMBOXANE $B_2$ LEVELS IN THE SERUM OF AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES



## FIGURE 38

THE THROMBOXANE $B_2$ LEVELS IN THE SERUM OF NON AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

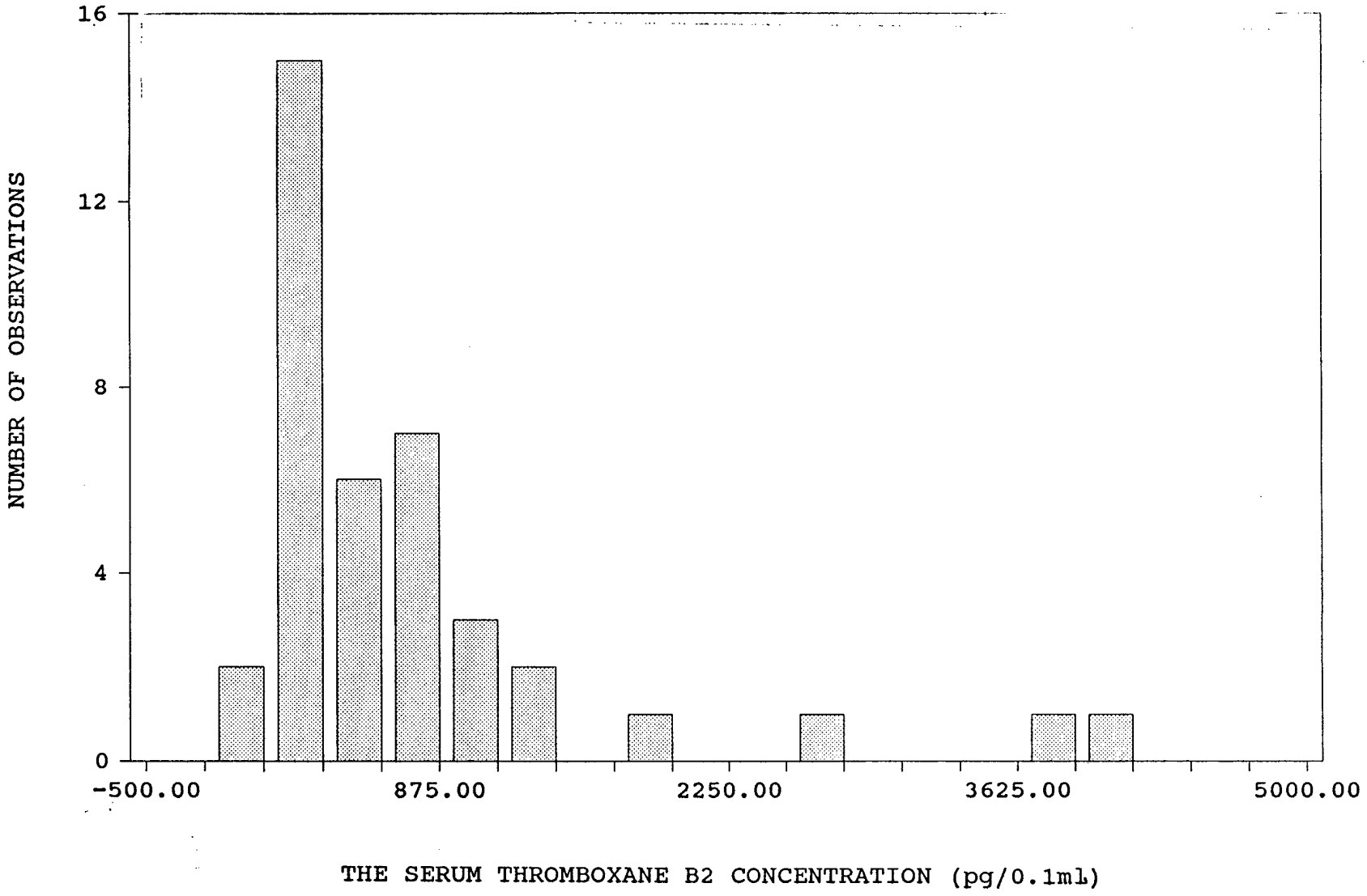


TABLE 14B

**THE NATURAL LOGARITHM OF THE IN VITRO CLOTTING TIME AND THROMBOXANE $B_2$ LEVEL IN THE SERUM OBTAINED FROM AGITATED OR NON-AGITATED WHOLE BLOOD CLOTTED AT FOUR TEMPERATURES**

| Waterbath Temp | 37C | 32C | 28C | 22C | 1 Way AOV |
|---|---|---|---|---|---|
| Clotting Time (min) | | | | | |
| Mean | 1.24 | 1.4 | 1.74 | 2.28 | <.0001 |
| SD | .3 | .3 | .3 | .6 | |
| n | 16 | 16 | 16 | 16 | |
| Serum TXB2 pg/0.1 ml from agitated whole blood | | | | | |
| Mean | 8.7 | 7.7 | 6.7 | 5.8 | <.0001 |
| SD | 1.0 | 1.0 | 1.1 | 1.4 | |
| n | 16 | 16 | 16 | 16 | |
| Serum TXB2 pg/0.1 ml from non-agitated whole blood | | | | | |
| Mean | 6.9 | 6.3 | 6.1 | 5.1 | <.01 |
| SD | .8 | .8 | .8 | 1.0 | |
| n | 10 | 10 | 10 | 10 | |
| Paired T between agitated whole blood and non-agitated whole blood | <.001 | <.001 | NS | NS | |

FIGURE 39

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE CLOTTING TIME IN AGITATED WHOLE BLOOD AND FOUR BLOOD TEMPERATURES

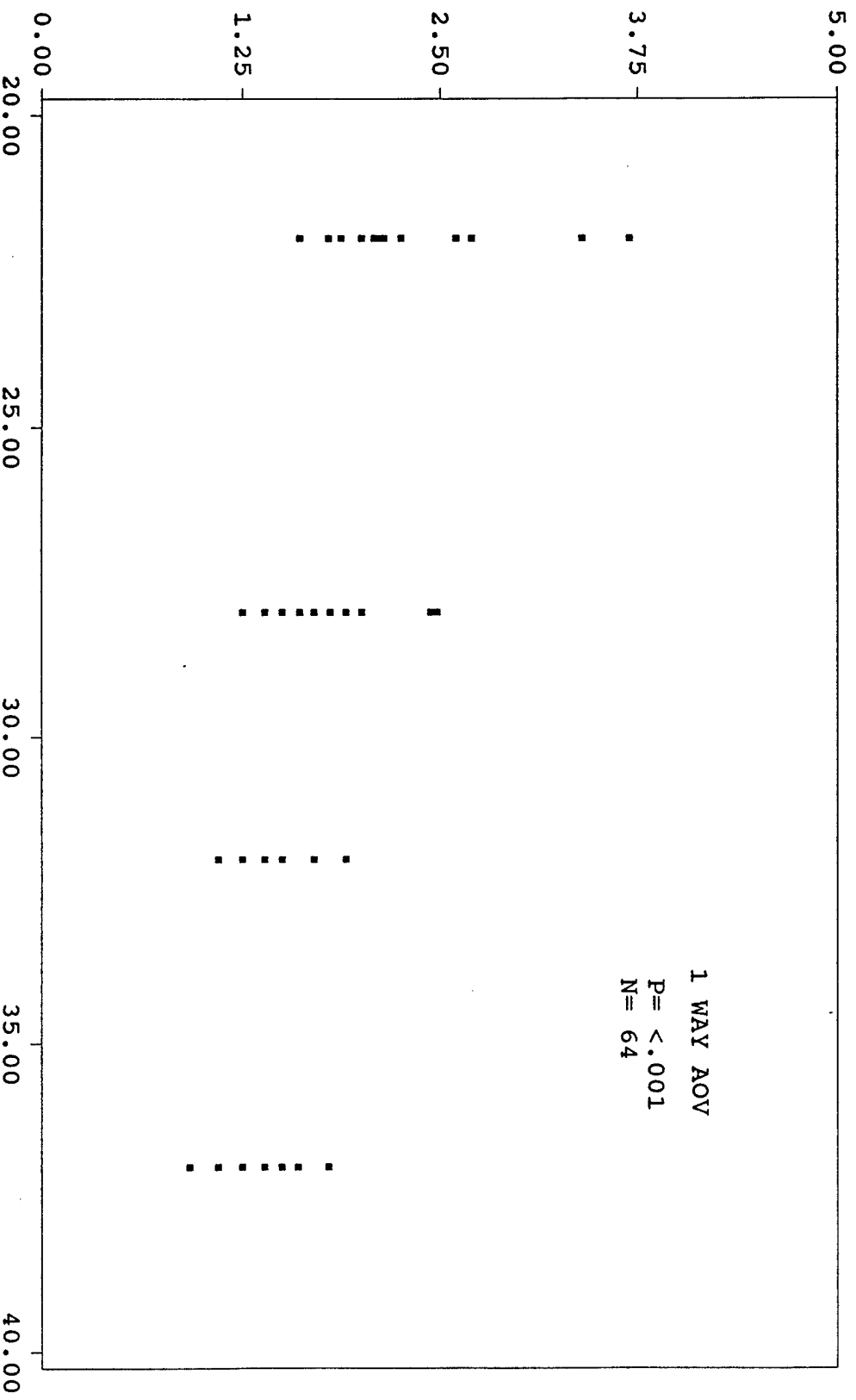
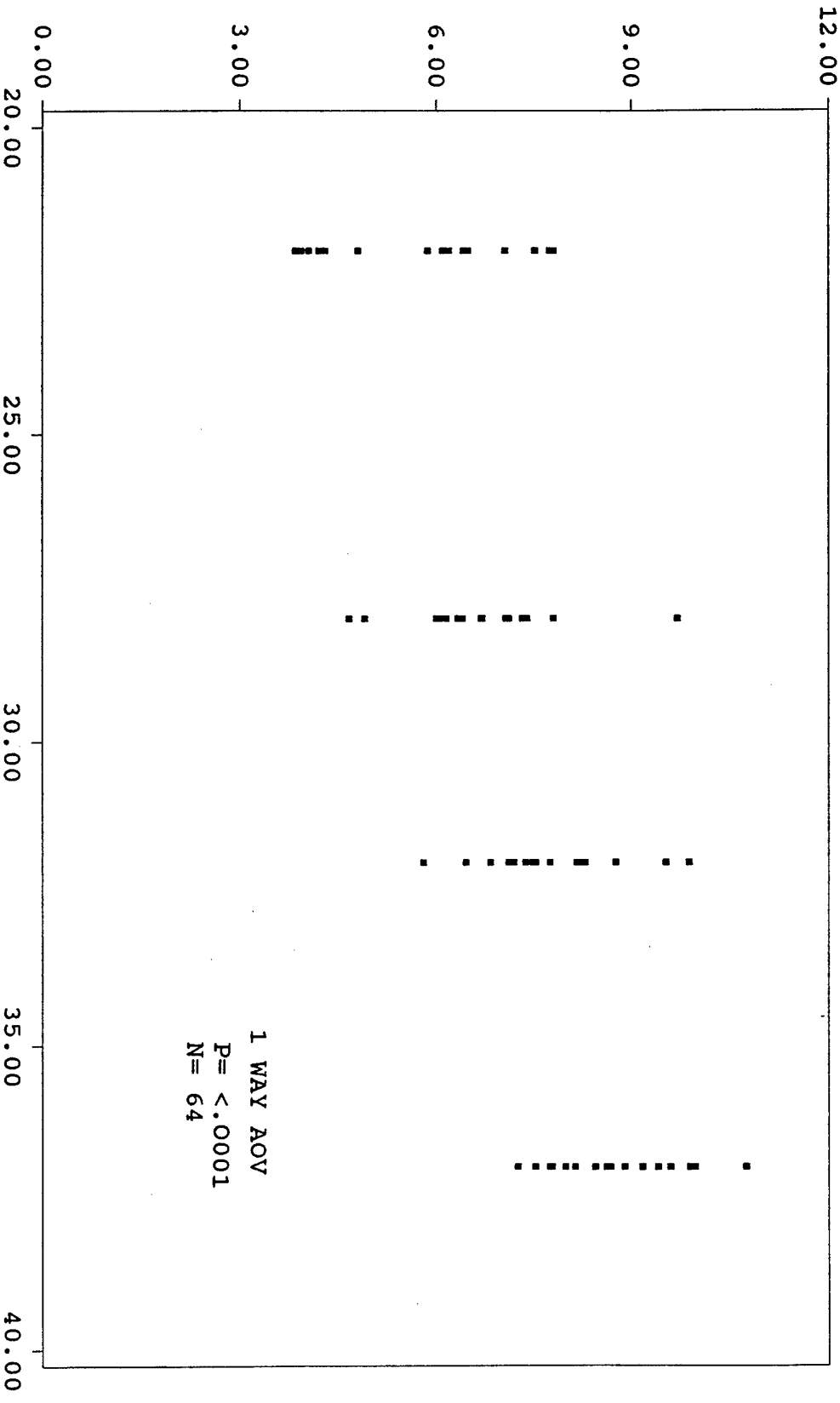


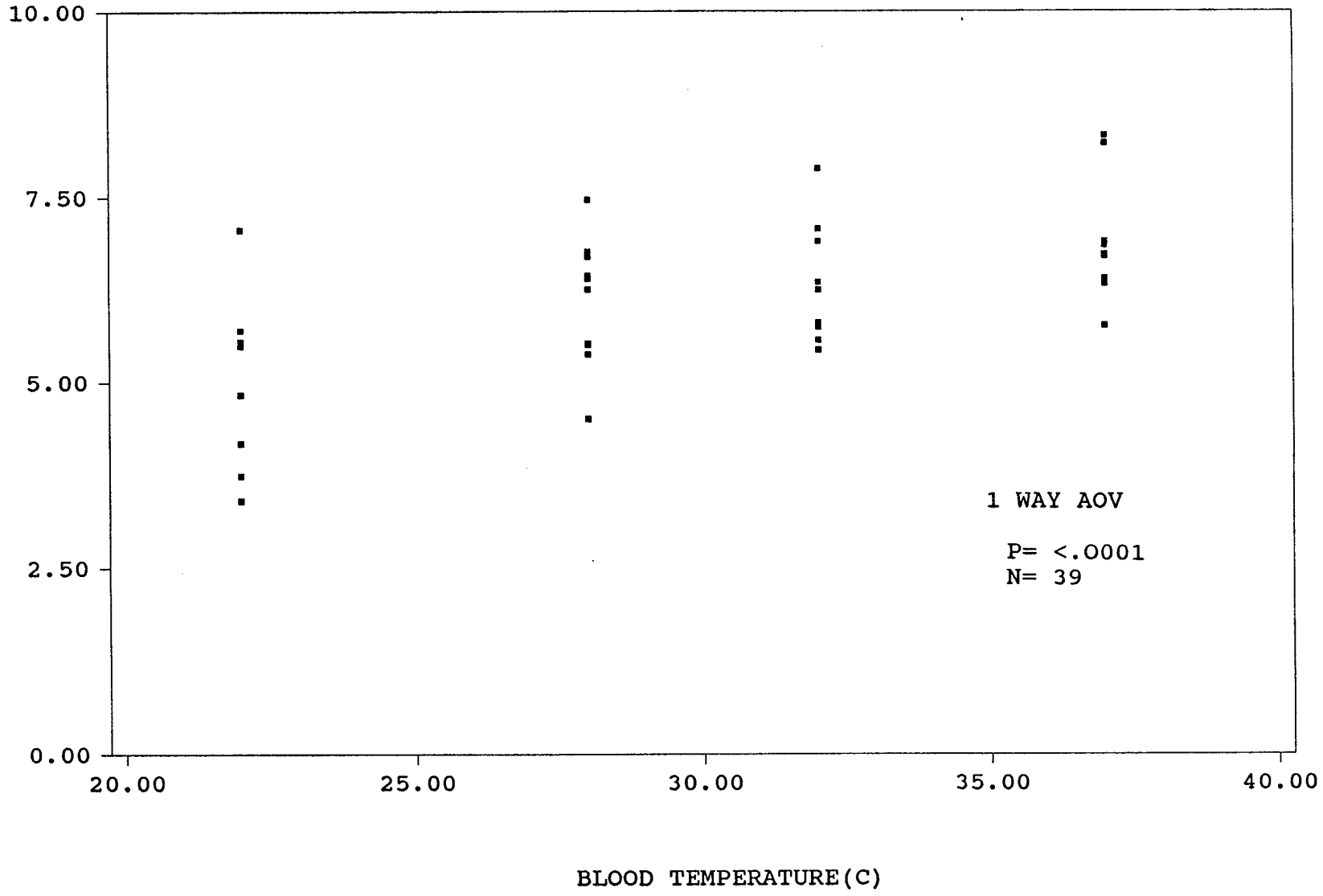
FIGURE 40

THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE $B_2$ LEVEL IN AGITATED CLOTTED BLOOD AT FOUR BLOOD TEMPERATURES



## FIGURE 41

**THE RELATIONSHIP BETWEEN THE NATURAL LOGARITHM OF THE SERUM THROMBOXANE $B_2$ LEVELS IN NON AGITATED CLOTTED BLOOD AND FOUR BLOOD TEMPERATURES**



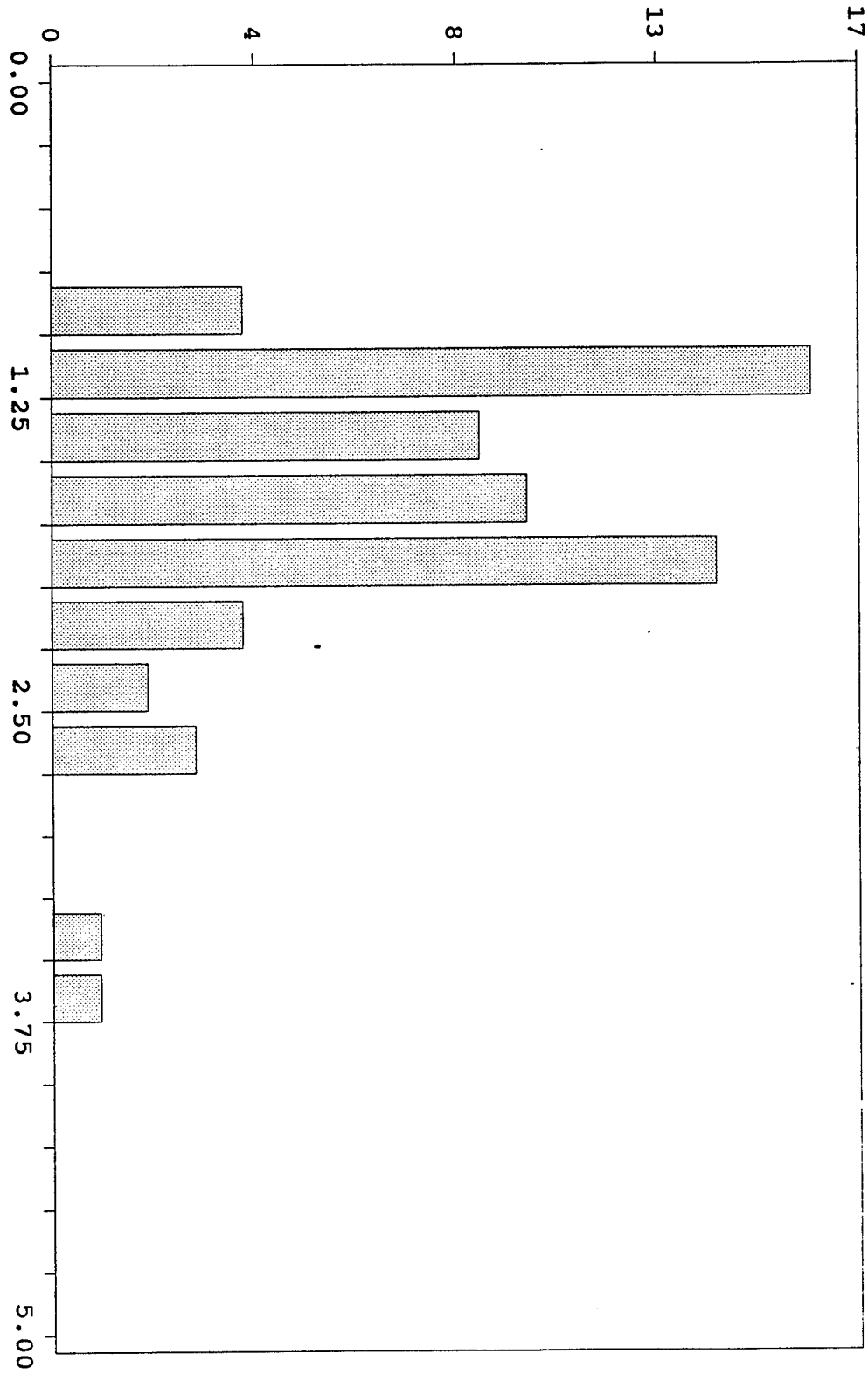
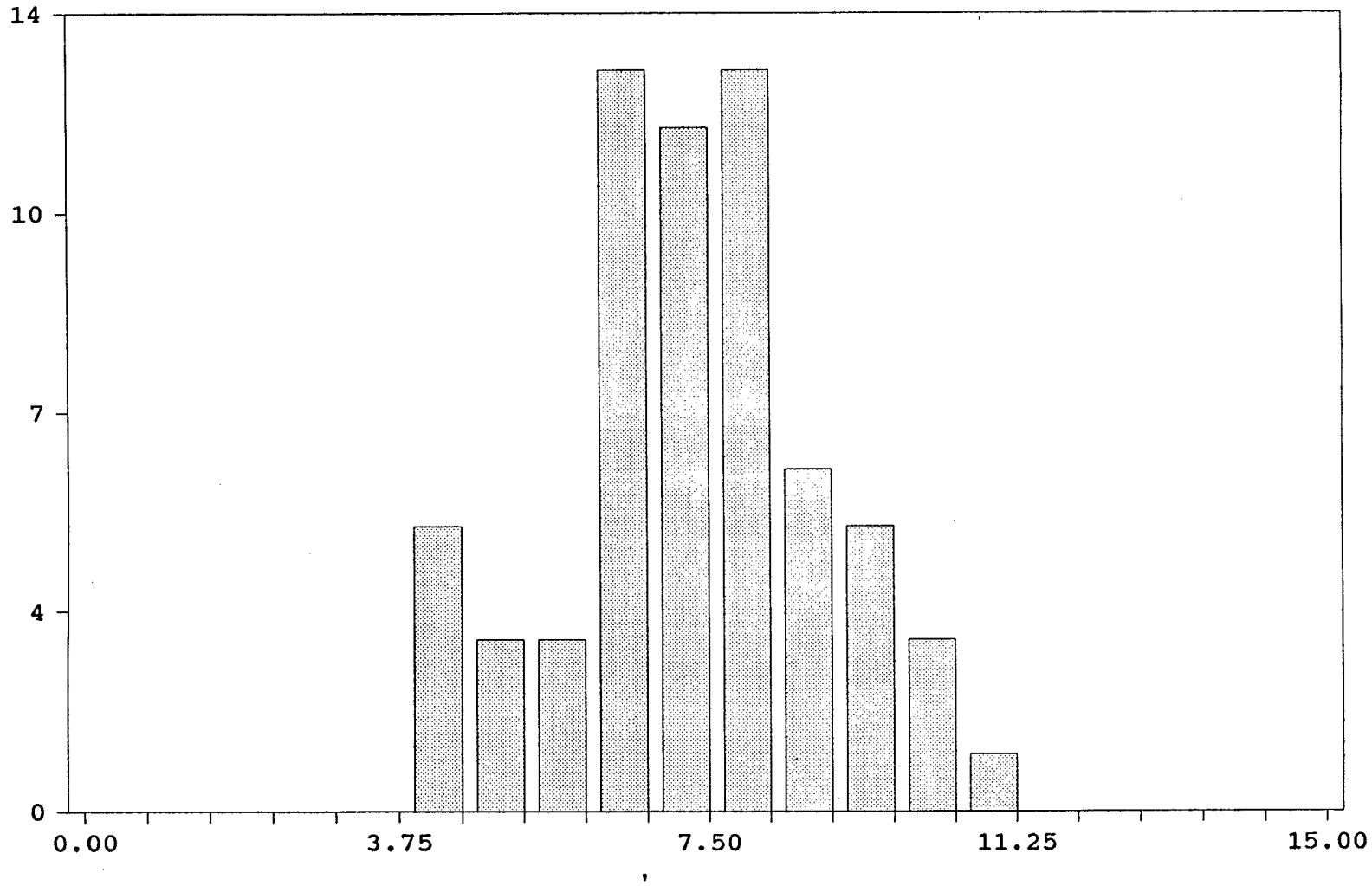



FIGURE 42

THE NATURAL LOGARITHM OF THE CLOTTING TIME OF AGITATED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

FIGURE 43

THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN SERUM OF AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES



## FIGURE 44

THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL IN THE SERUM OF NON AGITATED CLOTTED WHOLE BLOOD AT FOUR BLOOD TEMPERATURES

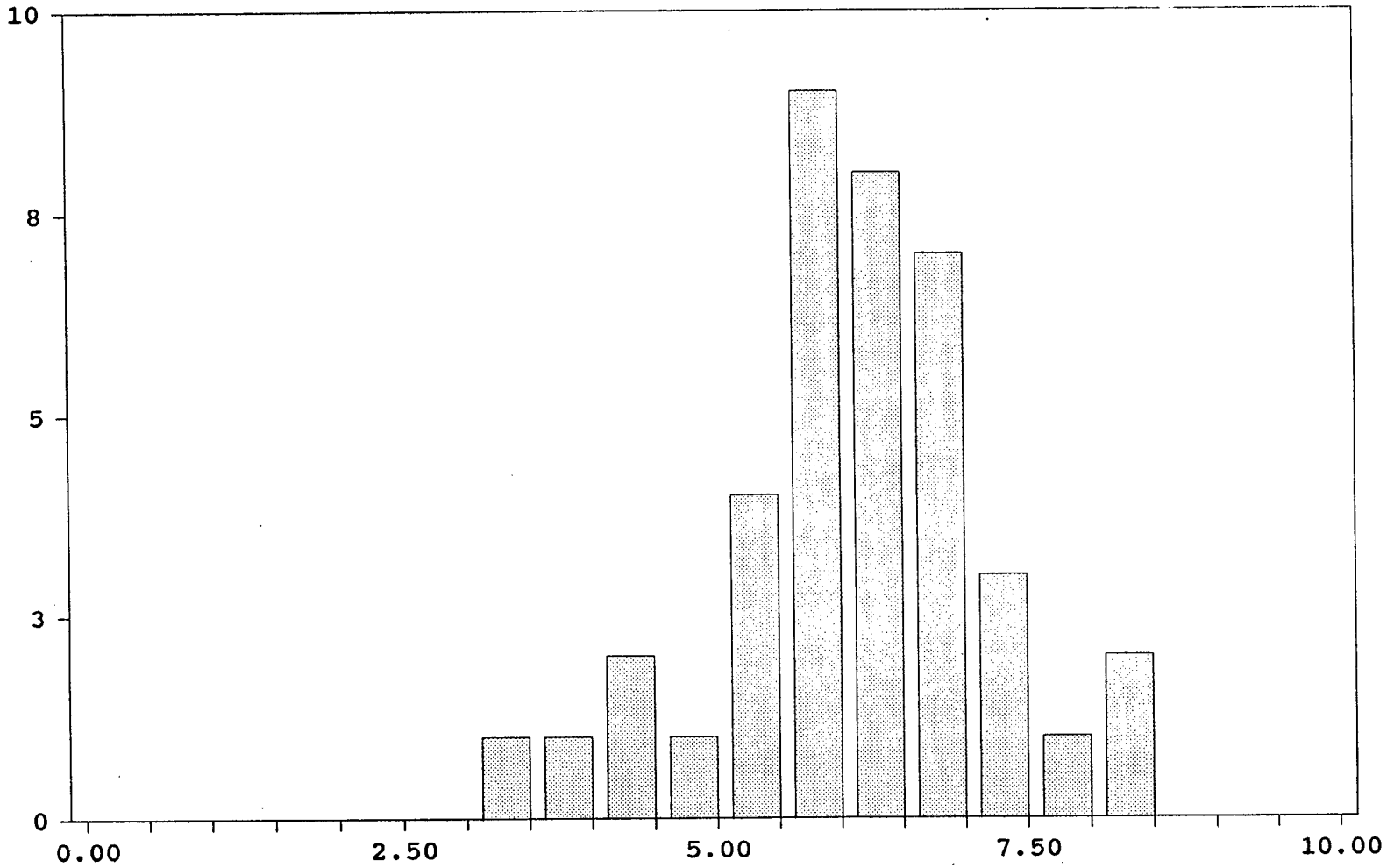


TABLE 15A

**THROMBOXANE $B_2$ LEVELS AT 30 SECOND INTERVALS IN THE SERUM SEPARATED FROM CLOTTED BLOOD MAINTAINED AT FOUR TEMPERATURES; ONE ML OF BLOOD AS COLLECTED INTO A 3.5 ML PLASTIC TUBE AND AGITATED EVERY 30 SECONDS**

**N=3**

**TIME IN MINUTES**

| Temp | | 30′ | 1 Min | 1′30" | 2′ | 2′30" | 1 WAY ANOVA |
|---|---|---|---|---|---|---|---|
| | | *TXB2 Level (pg/0.1 ml)* | | | | | |
| **37C** | | | | | | | |
| | 1. | 64 | 365 | 648 | 869 | 2696 | |
| | 2. | 329 | 207 | 767 | 2761 | 6765 | |
| | 3. | 56 | 100 | 143 | 351 | 747 | |
| | Mean: | 150 | 224 | 519 | 1327 | 3403 | NS |
| | SD: | 155 | 133 | 331 | 1268 | 3070 | |
| **32 C** | | | | | | | |
| | 1. | 277 | 751 | 1464 | 927 | 2613 | |
| | 2. | 227 | 824 | 1383 | 1380 | 1585 | |
| | 3. | 138 | 112 | 630 | 998 | 1160 | |
| | Mean: | 214 | 562 | 1159 | 1102 | 1786 | <.05 |
| | SD: | 70 | 392 | 460 | 244 | 747 | |
| **28 C** | | | | | | | |
| | 1. | 37 | 148 | 95 | 188 | 307 | |
| | 2. | 50 | 102 | 37 | 57 | 257 | |
| | 3. | 22 | 54 | 48 | 90 | 72 | |
| | Mean: | 36 | 101 | 60 | 112 | 212 | NS |
| | SD: | 14 | 47 | 31 | 68 | 124 | |
| **22 C** | | | | | | | |
| | 1. | 46 | 75 | 128 | 104 | 235 | |
| | 2. | 34 | 48 | 37 | 57 | 33 | |
| | 3. | 29 | 29 | 61 | 36 | 49 | |
| | Mean: | 36 | 51 | 75 | 66 | 106 | NS |
| | SD: | 9 | 23 | 47 | 35 | 112 | |
| 1 Way ANOVA: | | NS | NS | <.01 | NS | NS | |

TABLE 15B

**NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVEL AT 30 SECOND INTERVALS IN THE SERUM SEPARATED FROM CLOTTED BLOOD MAINTAINED AT FOUR TEMPERATURES; ONE ML OF BLOOD WAS COLLECTED INTO A 3.5 ML PLASTIC TUBE AND AGITATED EVERY 30 SECONDS**

n=3

TIME IN MINUTES

| Temp | | 30′ | 1 Min | 1′30" | 2′ | 2′30" | 1 Way ANOVA |
|---|---|---|---|---|---|---|---|
| | | *TXB2 Level (pg/0.1 ml)* | | | | | |
| **37C** | | | | | | | |
| | 1. | 4.16 | 5.9 | 6.47 | 6.77 | 7.9 | |
| | 2. | 5.8 | 5.53 | 6.64 | 7.92 | 8.82 | |
| | 3. | 4.03 | 4.61 | 4.96 | 5.86 | 6.62 | |
| | Mean: | 4.66 | 5.28 | 6.03 | 6.85 | 7.78 | <.05 |
| | SD: | 1.0 | .7 | .9 | 1.0 | 1.1 | |
| **32 C** | | | | | | | |
| | 1. | 5.62 | 6.62 | 7.29 | 6.83 | 7.87 | |
| | 2. | 5.42 | 6.71 | 7.23 | 7.23 | 7.37 | |
| | 3. | 4.93 | 4.72 | 6.45 | 6.91 | 7.06 | |
| | Mean: | 5.33 | 6.02 | 6.99 | 6.99 | 7.43 | <.01 |
| | SD: | .4 | 1.1 | .5 | .2 | .4 | |
| **28 C** | | | | | | | |
| | 1. | 3.61 | 5.0 | 4.55 | 5.24 | 5.73 | |
| | 2. | 3.91 | 4.62 | 3.61 | 4.04 | 5.55 | |
| | 3. | 3.09 | 3.99 | 3.87 | 4.5 | 4.28 | |
| | Mean: | 3.54 | 4.54 | 4.01 | 4.59 | 5.18 | <.05 |
| | SD: | .4 | .5 | .5 | .6 | .8 | |
| **22 C** | | | | | | | |
| | 1. | 3.83 | 4.32 | 4.85 | 4.64 | 5.46 | |
| | 2. | 3.53 | 3.87 | 3.61 | 4.04 | 3.5 | |
| | 3. | 3.37 | 3.37 | 4.11 | 3.58 | 3.89 | |
| | Mean: | 3.57 | 3.85 | 4.19 | 4.09 | 4.28 | NS |
| | SD: | .23 | .5 | .6 | .5 | 1.0 | |
| 1 Way ANOVA: | | <.05 | <.05 | <.01 | <.01 | <.01 | |

## TABLE 16

**THE RATE OF THE THROMBOXANE $B_2$ PRODUCTION AND THE NATURAL LOGARITHM OF THE RATE OF THE THROMBOXANE $B_2$ PRODUCTION IN BLOOD CLOTTED WITH AGITATION AT 37C, 32C, 28C, AND 22C AND IN SHED BLOOD FROM THE BLEEDING TIME SITE WHERE THE FOREARM TEMPERATURE WAS 32C, 28C, AND 22C**

| EMPERATURE degrees C) | RATE OF THROMBOXANE PRODUCTION (PG/ML/SECOND) | | NATURAL LOG OF THE RATE OF THROMBOXANE PRODUCTION (PG/ML/SECOND) | |
|---|---|---|---|---|
| | clotted blood N=16 | shed blood N=6 | clotted blood N=16 | shed blood N=6 |
| 37 | | | | |
| MEAN | 525 | | 5.7 | |
| SE | 162 | | 1.1 | |
| 32 | | | | |
| MEAN | 175 | 21.2 | 4.5 | 2.8 |
| SE | 63 | 6.5 | 1.1 | .7 |
| 28 | | | | |
| MEAN | 69 | 7.49 | 3.2 | 1.8 |
| SE | 40 | 2.0 | 1.5 | .7 |
| 22* | | | | |
| MEAN | 17 | .9 | 1.7 | .4 |
| SE | 5 | .3 | 1.0 | .9 |
| WAY AOV .0001 | <.01 | <.05 | <.001 | <.001 |

N=5

FIGURE 45

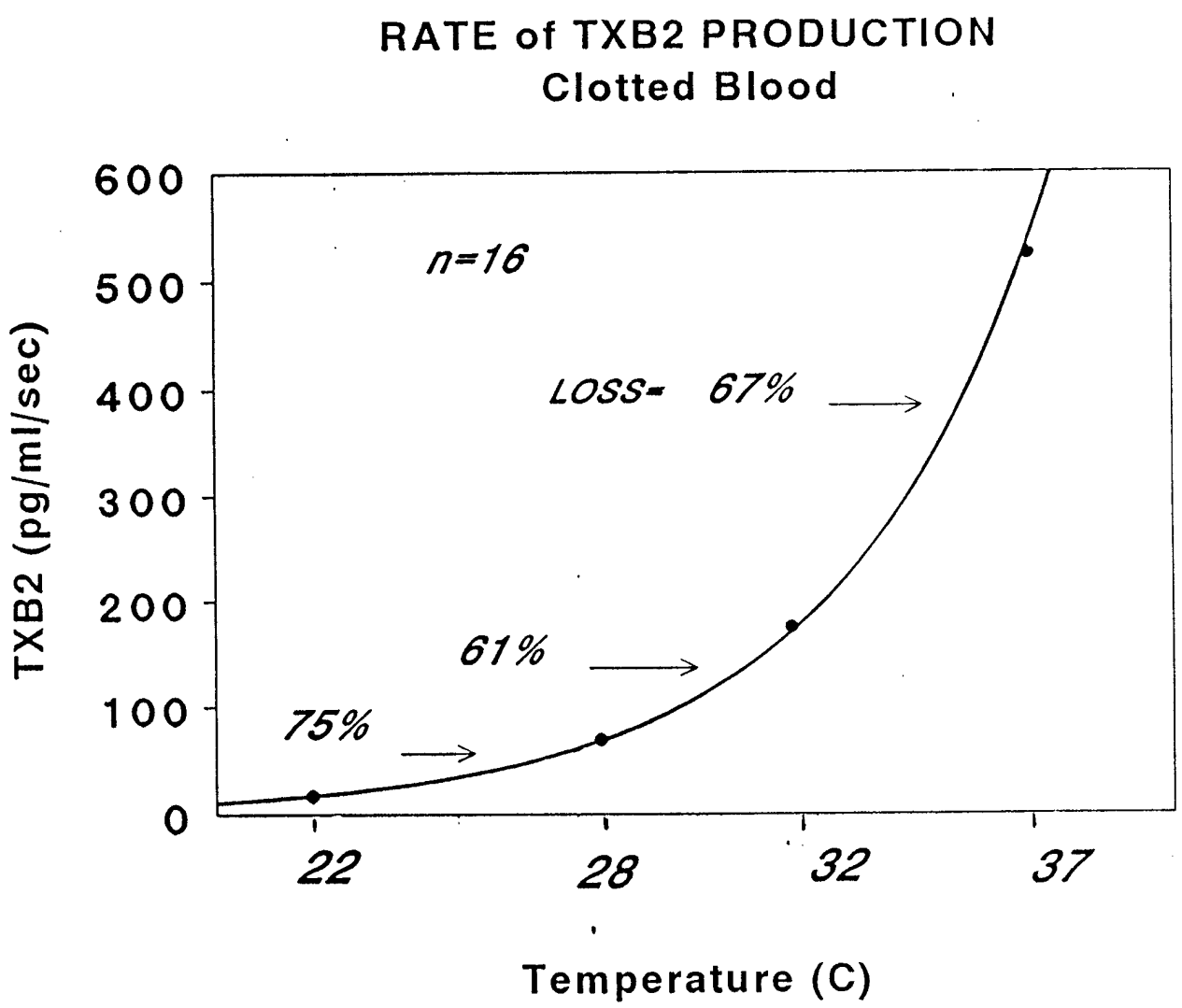
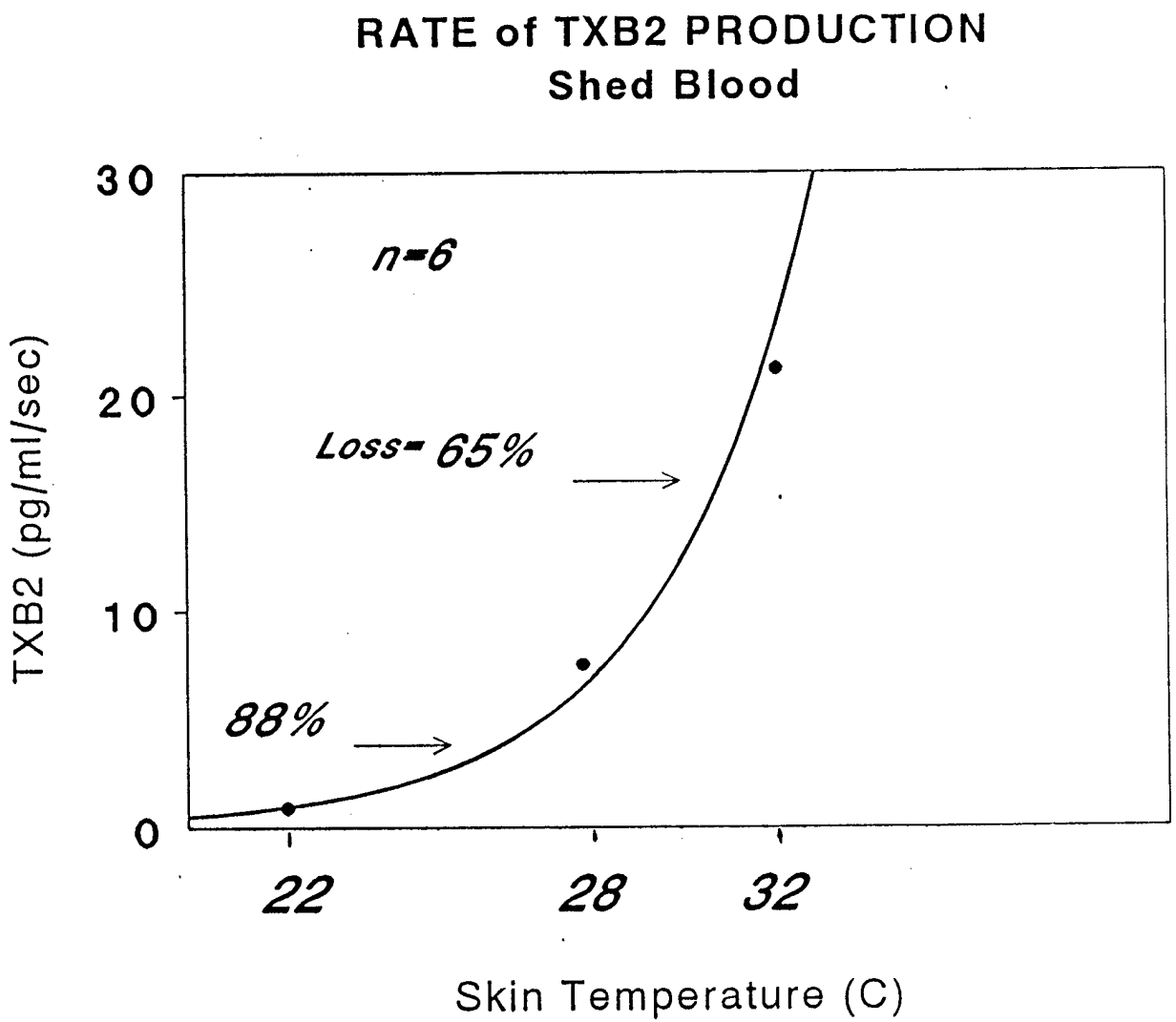

RATE of TXB2 PRODUCTION
Clotted Blood
TXB2 (pg/ml/sec)
600
500
400
300
200
100
0
n=16
LOSS- 67%
61%
75%
22
28
32
37
Temperature (C)

FIGURE 46


RATE of TXB2 PRODUCTION
Shed Blood
30
20
10
0
TXB2 (pg/ml/sec)
n=6
Loss= 65%
88%
22
28
32
Skin Temperature (C)

## FIGURE 47

THE RATE OF THROMBOXANE $B_2$ PRODUCTION IN AGITATED CLOTTED BLOOD MAINTAINED AT +22C, +28C, +32C, AND +37C

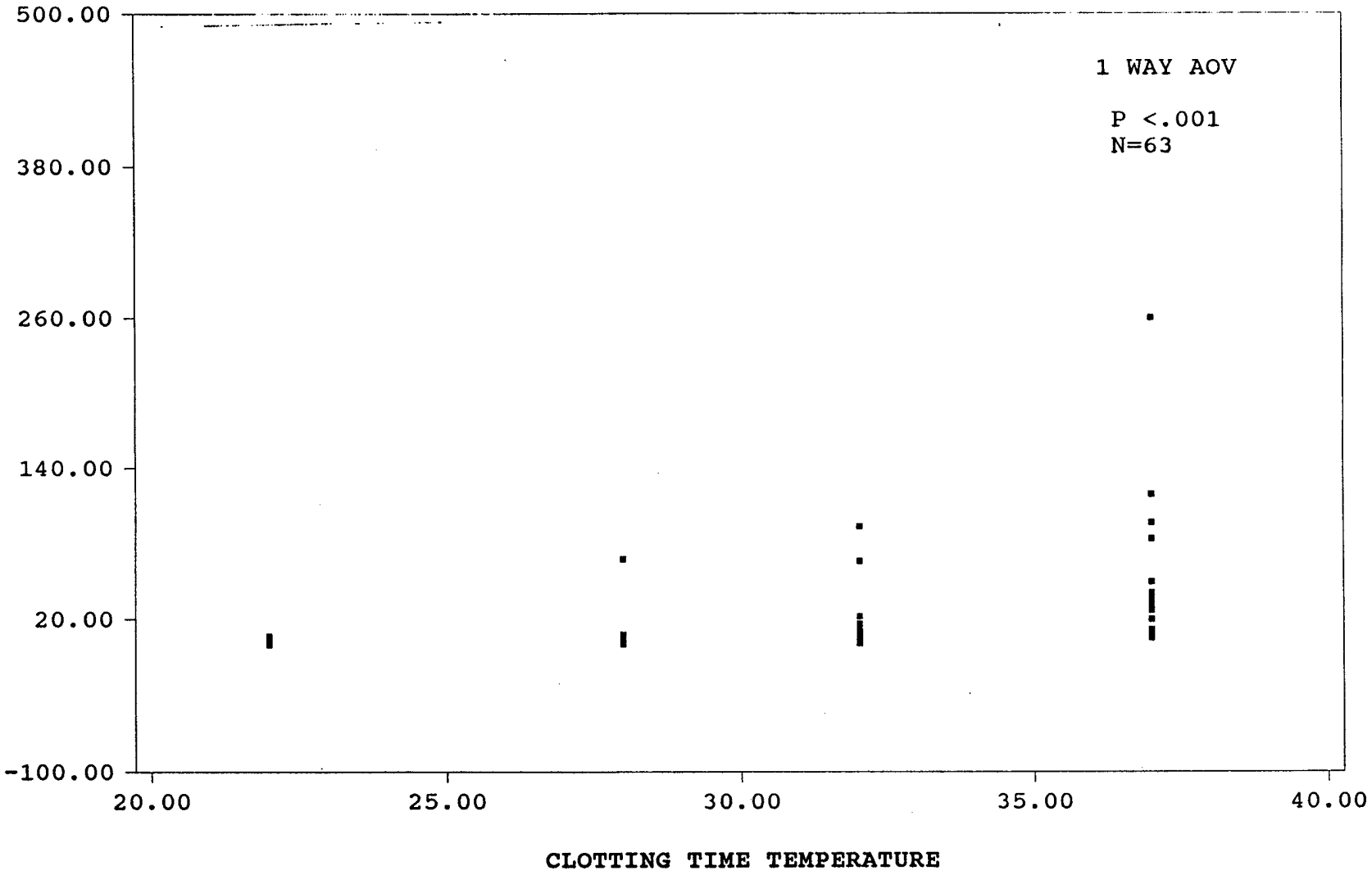


**FIGURE 48**

**THE NATURAL LOGARITHM OF THE RATE OF THE THROMBOXANE $B_2$ PRODUCTION IN BLOOD CLOTTED WITH AGITATION AT 22C, 28C, 32C, AND 37C**

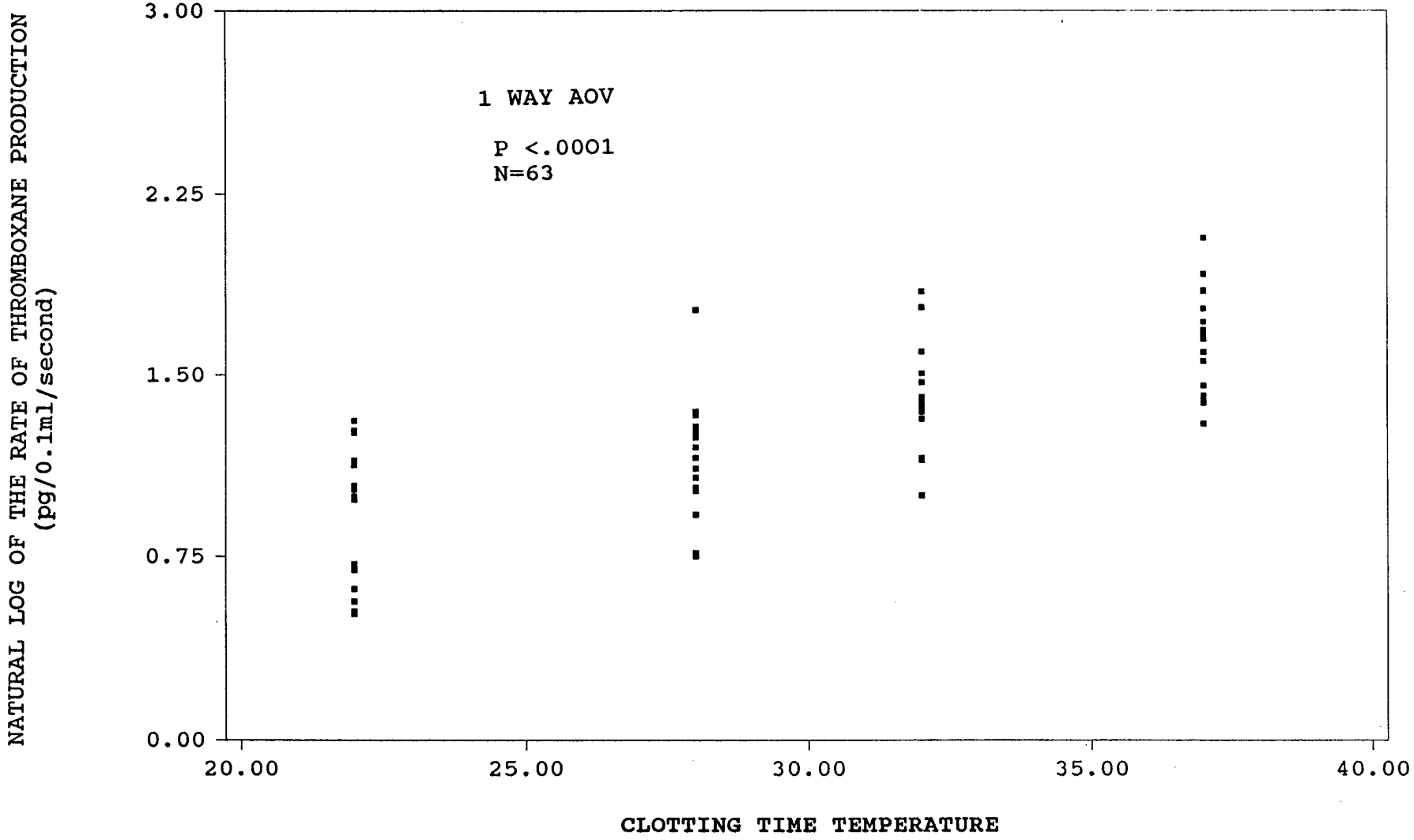


TABLE 17A

**BLEEDING TIME AND TOTAL HEMOGLOBIN IN THE SHED BLOOD COLLECTED ON THE FILTER PAPER IN 16 NORMAL VOLUNTEERS AT SEVEN SKIN TEMPERATURES**

| Temperature (C) | Bleeding Time (Min) | Total Hemoglobin Collected | Correlation Coefficient R | P |
|---|---|---|---|---|
| 38 | | | | |
| Mean: | 5.3 | 82 | | |
| SD: | 1.5 | 62 | | |
| n: | 16 | 16 | .764 | <.01 |
| 35 | | | | |
| Mean: | 5.3 | 61 | | |
| SD: | 1.6 | 43 | | |
| n: | 16 | 16 | .763 | <.05 |
| 32 | | | | |
| Mean: | 6.5 | 50 | | |
| SD: | 1.9 | 65 | | |
| n: | 16 | 16 | .740 | <.01 |
| 29 | | | | |
| Mean: | 10.5 | 71 | | |
| SD: | 2.8 | 36 | | |
| n: | 16 | 16 | .070 | NS |
| 26 | | | | |
| Mean: | 12.0 | 73 | | |
| SD: | 3.6 | 33 | | |
| n: | 16 | 16 | .574 | NS |
| 23 | | | | |
| Mean: | 19.5 | 83 | | |
| SD: | 6.7 | 51 | | |
| n: | 16 | 16 | .021 | NS |
| 20 | | | | |
| Mean: | 22.0 | 109 | | |
| SD: | 5.0 | 73 | | |
| n: | 10 | 10 | .642 | <.05 |
| All Temps 1 WAY AOV | <.001 | NS | 0.352 | <.001 |

FIGURE 49

THE RELATIONSHIP BETWEEN THE BLEEDING TIME AND THE TOTAL HEMOGLOBIN ON THE FILTER PAPER

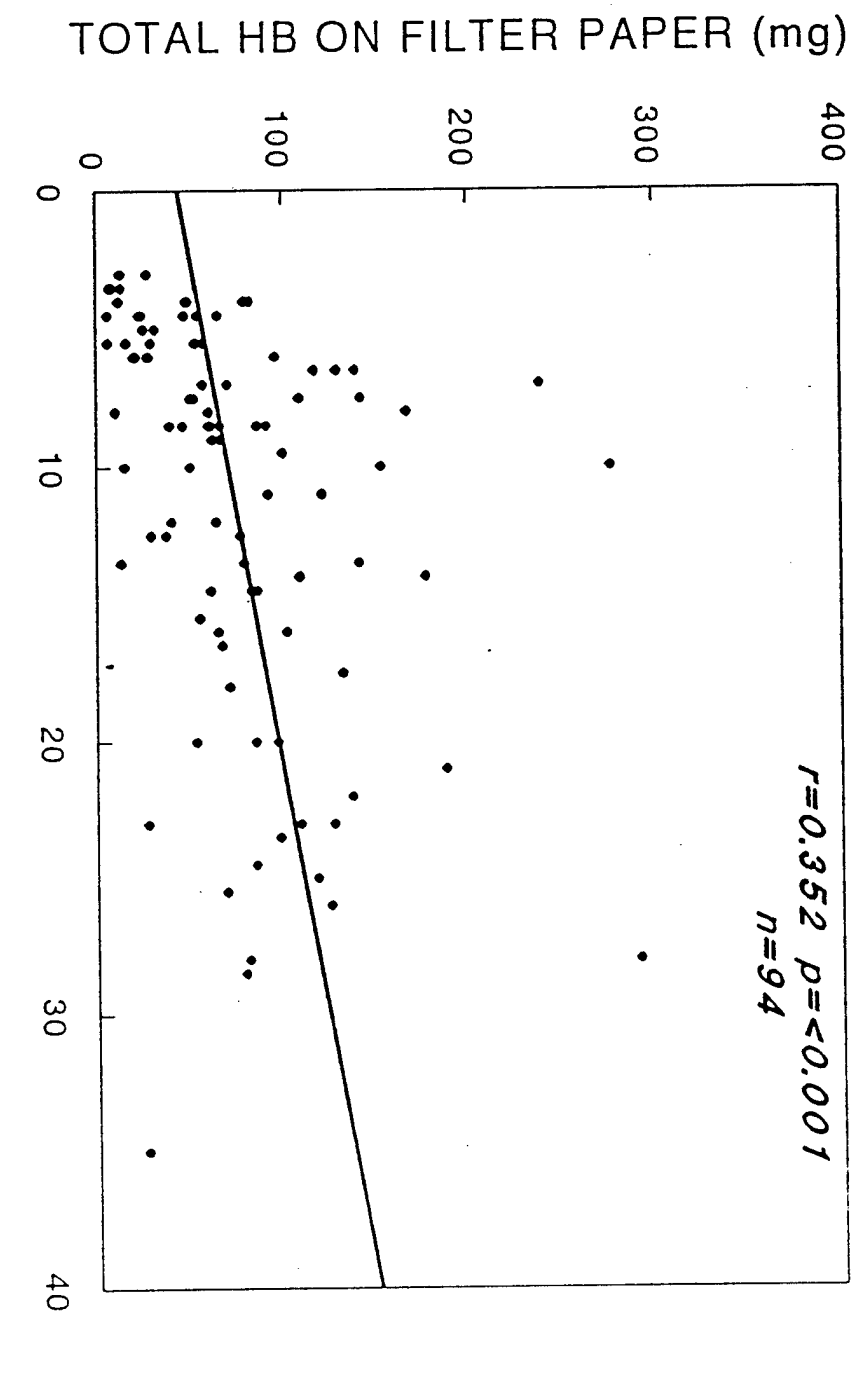


TABLE 17B

**NATURAL LOGARITHM OF THE BLEEDING TIME AND THE NATURAL LOGARITHM OF THE TOTAL HEMOGLOBIN IN THE SHED BLOOD COLLECTED ON THE FILTER PAPER IN 16 NORMAL VOLUNTEERS AT SEVEN SKIN TEMPERATURES**

| Temperature (C) | Bleeding Time (min) | Total Hemoglobin Collected | Correlation Coefficient R | P |
|---|---|---|---|---|
| 38 | | | | |
| Mean: | 1.62 | 4.11 | .734 | <.01 |
| SD: | .3 | .9 | | |
| 35 (n=10) | | | | |
| Mean: | 1.62 | 3.84 | .841 | <.01 |
| SD: | .3 | .8 | | |
| 32 | | | | |
| Mean: | 1.83 | 3.44 | .901 | <.001 |
| SD: | .3 | .9 | | |
| 29 | | | | |
| Mean: | 2.32 | 4.15 | .292 | NS |
| SD: | .3 | .5 | | |
| 26 (n=10) | | | | |
| Mean: | 2.44 | 4.04 | .801 | <.01 |
| SD: | .3 | .9 | | |
| 23 | | | | |
| Mean: | 2.92 | 4.15 | .311 | NS |
| SD: | .4 | .9 | | |
| 20 (n=10) | | | | |
| Mean: | 3.09 | 4.48 | .882 | <.01 |
| SD: | .3 | .8 | | |
| All Temps 1 Way AOV: | <.001 | NS | .493 | <.001 |

FIGURE 50

THE NATURAL LOGARITHM OF THE BLEEDING TIME AND
THE NATURAL LOGARITHM OF THE TOTAL HEMOGLOBIN ON THE FILTER PAPER

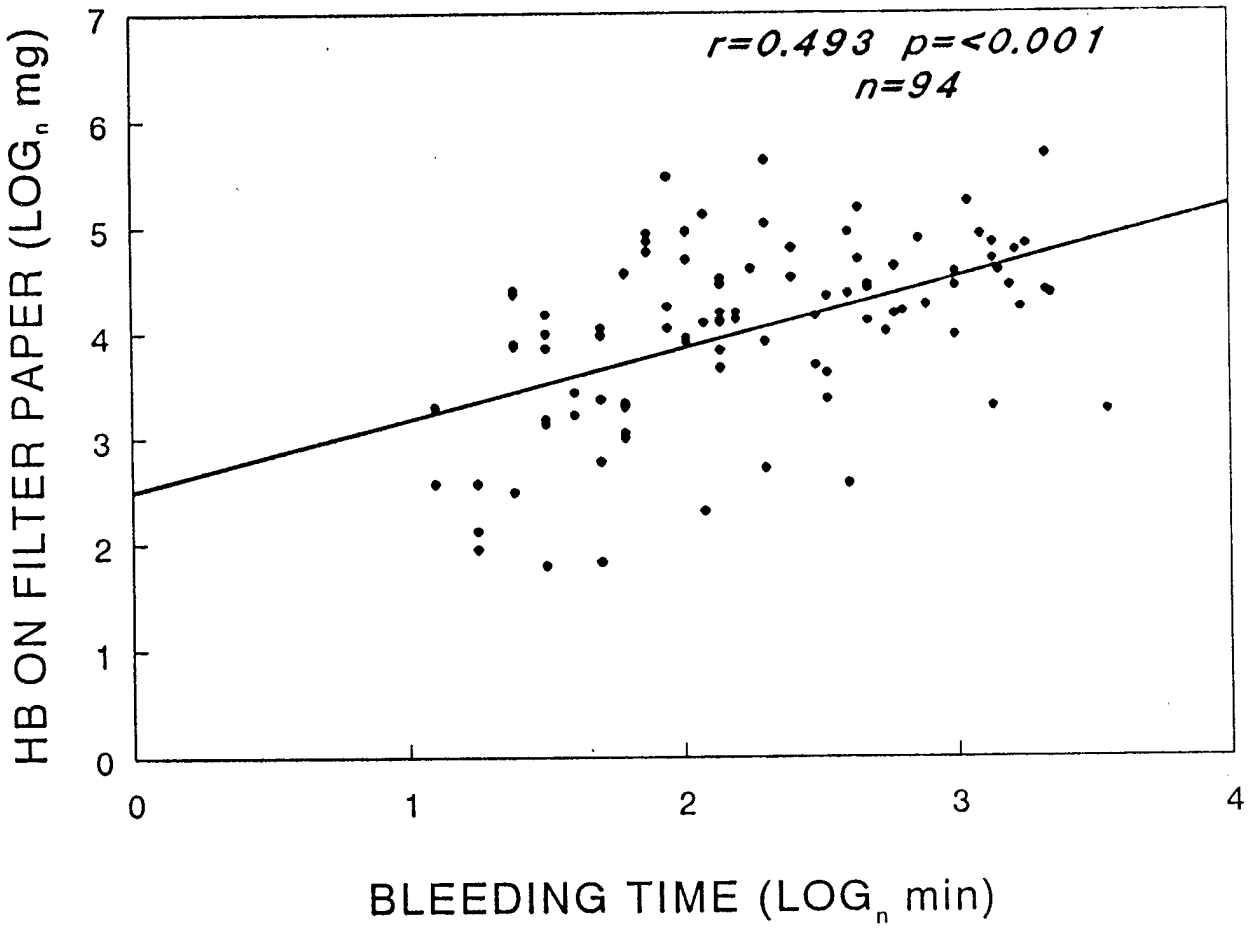


TABLE 18A

**DIGITIZED AGGREGATION PATTERNS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF 37C, 32C, 28C, AND 22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C**

| Temp | Aggregation to AA & ADP (digitized units/5 min) 0.05 mg/ml) (0.01 mM) | Ristocetin (digitized units/5min) (1.25 mg/ml) | Paired t Between AA & ADP and Ristocetin |
|---|---|---|---|
| **32C** | | | |
| Mean: | 273 | 244 | NS |
| SD: | 61 | 102 | |
| n: | 16 | 10 | |
| **37C** | | | |
| Mean: | 283 | 242 | NS |
| SD: | 68 | 109 | |
| n: | 16 | 11 | |
| **28C** | | | |
| Mean: | 251 | 283 | NS |
| SD: | 67 | 57 | |
| n: | 16 | 10 | |
| **22C** | | | |
| Mean: | 261 | 234 | NS |
| SD: | 89 | 99 | |
| n: | 16 | 10 | |
| 1 Way ANOVA: NS | NS | NS | |

TABLE 18B

**THE NATURAL LOGARITHM OF THE DIGITIZED AGGREGATION PATTERNS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF 37C, 32C, 28C, AND 22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C**

| Temp | Aggregation to AA and ADP (digitized units/5 min) (0.05 mg/ml) (0.01 mM) | Ristocetin (digitized units/5min) (1.25 mg/ml) | Paired t Between AA & ADP and Ristocetin |
|---|---|---|---|
| **32C** | | | |
| Mean: | 5.58 | 5.39 | NS |
| SD: | .3 | .5 | |
| n: | 16 | 10 | |
| **37C** | | | |
| Mean: | 5.62 | 5.37 | NS |
| SD: | .2 | .6 | |
| n: | 16 | 11 | |
| **28C** | | | |
| Mean: | 5.48 | 5.62 | NS |
| SD: | .3 | .2 | |
| n: | 16 | 10 | |
| **22C** | | | |
| Mean: | 5.53 | 5.31 | NS |
| SD: | .3 | .7 | |
| n: | 16 | 10 | |
| 1 Way ANOVA: | NS | NS | |

TABLE 19A

**THROMBOXANE $B_2$ LEVELS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF +37C, +32C, +28C, AND +22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C**

| Temp | AA (0.05 mg/ml) & ADP (0.01 mM) TxB2 Production Per Plt X10-5 (pg/0.1 ml) | Ristocetin (1.25 mg/ml) TxB2 Production Per Plt X10-5 (pg/0.1 ml) | Non Paired t Test Between AA &ADP and Ristocetin |
|---|---|---|---|
| **32C** | | | |
| Mean: | 3.2 | .8 | <.01 |
| SD: | 1.2 | .8 | |
| n: | 6 | 5 | |
| **37C** | | | |
| Mean: | 2.9 | 1.0 | <.05 |
| SD: | 1.4 | 1.0 | |
| n: | 7 | 8 | |
| **28C** | | | |
| Mean: | 3.4 | 0.7 | <.01 |
| SD: | 1.8 | .6 | |
| n: | 7 | 6 | |
| **22C** | | | |
| Mean: | 3.12 | 1.1 | <.05 |
| SD: | 2.0 | .7 | |
| n: | 8 | 5 | |
| 1 way ANOVA: | NS | NS | |

TABLE 19B

**THE NATURAL LOGARITHM OF THE THROMBOXANE $B_2$ LEVELS FOLLOWING STIMULATION OF PLATELET RICH PLASMA OBTAINED FROM THE ANTECUBITAL VEIN OF THE FOREARM SUBJECTED TO LOCAL WARMING AND COOLING TO ACHIEVE TEMPERATURES OF +37C, +32C, +28C, AND +22C WITH A COMBINATION OF AA AND ADP OR WITH RISTOCETIN ALONE WHEN THE PLATELET RICH PLASMA WAS MAINTAINED AT 37C**

| Temp | AA (0.05 mg/ml) & ADP (0.01 mM) TxB2 Production Per Plt X10-5 (pg/0.1 ml) | Ristocetin (1.25 mg/ml) TxB2 Production Per Plt X10-5 (pg/0.1 ml) | Non Paired t Test Between AA &ADP and Ristocetin |
|---|---|---|---|
| **32C** | | | |
| Mean: | 1.1 | -1.3 | <.05 |
| SD: | .4 | 1.8 | |
| n: | 6 | 5 | |
| **37C** | | | |
| Mean: | .95 | -.7 | <.01 |
| SD: | .5 | 1.3 | |
| n: | 7 | 8 | |
| **28C** | | | |
| Mean: | 1.09 | -1.14 | <.05 |
| SD: | .5 | 1.6 | |
| n: | 7 | 6 | |
| **22C** | | | |
| Mean: | .82 | -.39 | NS |
| SD: | 1.0 | 1.3 | |
| n: | 8 | 5 | |
| 1 way ANOVA: | NS | NS | |

TABLE 20A

**DIGITIZED AGGREGATION PATTERNS AND IN VITRO THROMBOXANE $B_2$ PRODUCTION BY PLATELETS IN RESPONSE TO RISTOCETIN ALONE OR A COMBINATION OF ARACHADONIC ACID AND ADP AT 22C AND 37C IN PLATELET RICH PLASMA SEPARATED FROM WHOLE BLOOD COLLECTED IN SODIUM CITRATE**

N=5

*FINAL CONCENTRATION OF ARACHADONIC ACID (.05 MG/ML) AND ADP (.01 mM)*

| | Aggregation (digitized units/5min) 22C | 37C | Paired T 22-37 | TXB2 Prod per Plt ($\times 10^{-5}$) per 22C | 37C | Paired t 22-37 |
|---|---|---|---|---|---|---|
| Mean: | 237 | 265 | NS | 4.4 | 8.0 | NS |
| SD: | 103 | 58 | | 6.0 | 9.3 | |

*FINAL CONCENTRATION OF RISTOCETIN (1.25 MG/ML)*

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean: | 147 | 219 | | .07 | .99 | NS |
| SD: | 53 | 96 | NS | .07 | .96 | |
| Paired T Dual-Rist: | <.05 | NS | | NS | NS | |

TABLE 20B

**NATURAL LOGARITHM OF THE DIGITIZED AGGREGATION PATTERNS AND THROMBOXANE $B_2$ PRODUCTION BY PLATELETS IN RESPONSE TO RISTOCETIN ALONE OR A COMBINATION OF ARACHADONIC ACID AND ADP AT 22C AND 37C IN VITRO IN PLATELET RICH PLASMA SEPARATED FROM WHOLE BLOOD COLLECTED IN SODIUM CITRATE**

N=5

*FINAL CONCENTRATION OF ARACHADONIC ACID (.05 MG/ML) AND ADP (.01 mM)*

| | Aggregation (Digitized Units/5min) | | Paired T | TXB2 Prod per Plt ($x10^{-5}$) | | Paired |
|---|---|---|---|---|---|---|
| | 22C | 37C | 22-37 | 22C | 37C | 22-37 |
| Mean: | 5.4 | 5.6 | NS | 0.3 | 1.5 | NS |
| SD: | .5 | .2 | | 1.9 | 1.2 | |

*FINAL CONCENTRATION OF RISTOCETIN (1.25 MG/ML)*

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean: | 4.9 | 5.3 | | -2.9 | -.47 | |
| SD: | .4 | .5 | NS | .9 | 1.2 | <.01 |
| Paired T Dual-Rist: | <.05 | NS | | NS | NS | |

## TABLE 21

### THE MEASURED AND TEMPERATURE CORRECTED BLEEDING TIME IN EACH OF TEN NORMAL VOLUNTEERS

| SKIN TEMP: | 38C | | 35C | | 32C | | 29C | | 26C | | 23C | | 20C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Meas | Corr | Meas | Corr | Meas | Corr | Meas | Corr | Meas | Corr | Meas | Corr | Meas | Corr |
| DONOR # | | | | | | | | | | | | | | |
| 1 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.0 | 5.5 | 3.9 | 5.5 | 3.0 | 8.0 | 3.2 | 10.0 | 2.5 |
| 2 | 3.0 | 3.0 | 3.0 | 3.0 | 4.0 | 3.4 | 8.0 | 5.6 | 8.5 | 4.7 | 16.0 | 6.4 | 20.0 | 5.0 |
| 3 | 4.5 | 4.5 | 4.5 | 4.5 | 6.0 | 5.1 | 10.0 | 7.0 | 12.0 | 6.6 | 15.5 | 6.2 | 23.5 | 5.9 |
| 4 | 7.0 | 7.0 | 7.5 | 7.5 | 8.5 | 7.2 | 11.0 | 7.7 | 12.5 | 6.9 | 22.3 | 8.9 | 19.8 | 4.9 |
| 5 | 6.0 | 6.0 | 5.3 | 5.3 | 6.0 | 5.1 | 9.0 | 6.3 | 14.0 | 7.7 | 17.5 | 7.0 | 25.0 | 6.3 |
| 6 | 8.0 | 8.0 | 7.5 | 7.5 | 7.5 | 6.4 | 12.0 | 8.4 | 12.5 | 6.9 | 13.5 | 5.4 | 23.0 | 5.8 |
| 7 | 7.0 | 7.0 | 6.5 | 6.5 | 9.5 | 8.0 | 11.0 | 7.7 | 14.5 | 8.0 | 23.0 | 9.2 | 24.5 | 6.1 |
| 8 | 7.5 | 7.5 | 6.5 | 6.5 | 10.0 | 8.5 | 10.0 | 7.0 | 14.0 | 7.7 | 21.0 | 8.4 | 28.0 | 7.0 |
| 9 | 4.0 | 4.0 | 4.0 | 4.0 | 6.0 | 5.1 | 8.5 | 6.0 | 8.5 | 4.7 | 16.0 | 6.4 | 28.0 | 7.0 |
| 10 | 4.0 | 4.0 | 4.5 | 4.5 | 4.5 | 3.8 | 8.5 | 6.0 | 18.0 | 9.9 | 20.0 | 8.0 | 20.0 | 5.0 |
| Mean: | 5.5 | 5.5 | 5.3 | 5.3 | 6.6 | 5.6 | 9.4 | 6.6 | 12.0 | 6.6 | 17.3 | 6.9 | 22.2 | 5.6 |
| SD: | 1.8 | 1.8 | 1.6 | 1.6 | 2.3 | 1.9 | 1.9 | 1.3 | 3.6 | 2.0 | 4.5 | 1.8 | 5.2 | 1.3 |
| n: | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Range: | 3.0-8.0 | 3.0-8.0 | 3.0-7.5 | 3.0-7.5 | 3.0-8.0 | 3.0-8.5 | 5.5-12.0 | 3.9-8.4 | 5.5-18.0 | 3.0-9.9 | 8.0-23.0 | 3.2-9.2 | 10.0-28.0 | 2.5-7.0 |

FIGURE 51

THE MEASURED AND CORRECTED BLEEDING TIME IN THE TEN (10) NORMAL VOLUNTEERS AT THE SEVEN (7) SKIN TEMPERATURES.